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**THE DEVESCOVINID FLAGELLATES
CADUCEIA THEOBROMAE FRANÇA
PSEUDODEVESCOVINA RAMOSA NEW
SPECIES AND MACROTRICHOMONAS
PULCHRA GRASSI**

**BY
HAROLD KIRBY, JR.**

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THE DEVESCOVINID FLAGELLATES CADUCEIA
THEOBROMAE FRANÇA,
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AND MACROTRICHOMONAS PULCHRA
GRASSI

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INTRODUCTION

THE SUBFAMILY DEVESCOVININAE, which is one of the largest and most diversified groups of flagellates in termites, has representatives in the families Mastotermitidae, Hodotermitidae, and Kalotermitidae. In the last-named family one or more species occur in each of all but five or six of more than a hundred host species examined. Most of these flagellates belong to the genera *Foaina*, *Devescovina*, and *Metadevescovina*. *Caduceia*, *Pseudodevescovina*, and *Macrotrichomonas* are not so rich in species, but the flagellates are particularly large and noteworthy. The characteristics of the type species of *Caduceia* and *Macrotrichomonas*, *C. theobromae* and *M. pulchra*, are poorly known, owing to the inadequacy of the descriptions by França and Grassi. Because the two species are very important in the comparative morphology and taxonomy of the Devescovininae, a complete description of them is particularly desirable.

The writer has published (1936) accounts of *Pseudodevescovina uniflagellata* Sutherland, the type species of its genus, and *Caduceia nova* (Grassi). In a footnote to that paper it was stated that a flagellate apparently identical with *Caduceia theobromae* França had been found in a termite in Tanganyika Territory. This flagellate is described here, as well as a new species of *Pseudodevescovina* from another species of termite in Tanganyika Territory.

Macrotrichomonas pulchra Grassi was found by the writer in 1925 in a termite in Costa Rica, *Kaloterme contracticornis*. The study of its structure and reproduction had been completed when a note was published (Kirby, 1933) on the fate of differentiated organelles in the division process in devescovinid flagellates. The figures of the division process in *M. pulchra*, which are reproduced in the present paper, were shown then at the meeting of the American Society of Zoölogists. Comparison with the type of the species was possible through the kindness of Professor F. Silvestri, who lent the writer a slide he had made from *Glyptotermes parvulus* at the time material was supplied to Grassi.

Grassé (1937) stated that he had studied *Caduceia theobromae*, *Macrotrichomonas pulchra*, and *Pseudodevescovina punctata* in African termites. The last-named is not a described species; evidently it is a *nomen nudum* for a new species found by him. He reported having observed in the three deves-

covinids the amputation and subsequent resorption of the posterior part of the parabasal body at the beginning of division, leaving in place a proximal part that is about the length of the nucleus; the origin of a new parabasal near, but apparently not in continuity with, the free pole of the spindle; and the subsequent growth of both parabasals. Grassé stated that, in describing a similar process in *Pseudodevescovina uniflagellata*, Kirby (1936) had found again the facts that he and Fauré (1935) discovered in *Trichomonas caviae*. A note on the occurrence of the same process in several species of devescovinids had been published, however, nearly four years before (Kirby, 1933), when Grassé was maintaining that the parabasal bodies of trichomonad and devescovinid flagellates reproduce by longitudinal fission (Duboscq and Grassé, 1933). The writer's statement (1933, p. 93) reads: "The proximal part of the parabasal, usually for about nuclear length, is retained. A new one differentiates to equal size, then both grow equally. The detached part of the parabasal is resorbed." These conclusions were derived from observations on *Pseudodevescovina uniflagellata*, *Macrotrichomonas pulchra*, and species of *Foaina*, *Devescovina*, and *Metadevescovina*.

The collection of material from Central America was aided by a grant from the Bache Fund of the National Academy of Sciences, given to Dr. L. R. Cleveland. The studies in Africa were made while the writer was a Fellow of the John Simon Guggenheim Memorial Foundation. The termites from Java and Ceylon in which *M. pulchra* was found were collected by Miss Jane Collier for Cleveland, and smears of the protozoa were given to the writer by him. Preparation of the report has been aided by grants from the National Research Council, the Research Board of the University of California, and the Works Progress Administration of the United States Government (Work Project 4540). The drawings were made under the writer's supervision by Miss Dorothy G. Harris and Mr. Carl Stover, and valuable technical assistance was given by Dr. Elmer R. Noble.

Caduceia França

Caduceia França, 1918, Bull. Soc. Port. Sci. Nat., 8:94; genotype *C. theobromae* França.

Diagnosis.—Devescovinids of large size, known species 46–124 μ long, averaging 62–90 μ , stout, bluntly pointed or rounded ends; three anterior flagella; trailing flagellum a slender cord, usually less than body length; cresta relatively small, length in known forms 1–12 μ ; chromatic shield, composed of small granules, present against one side of nucleus; parabasal body single, well developed, usually coiled tightly 3–15 times, in *C. theobromae* more loosely coiled; capitulum of axostyle a simple expansion, trunk tapered to a filamentous posterior portion usually entirely enclosed in cytoplasm; nucleus elongated longitudinally as in *Devescovina*, with well-defined membrane, peripheral clear space, and coarsely granular chromatin mass; adherent, slender, rod-shaped microorganisms producing apparent striations on restricted areas of the surface of some species, absent on others; all known species with an investment of short spirochaetes, usually 4–6 μ long, on most of the surface.

Koidzumi (1921), Cleveland (1923), Duboscq and Grassé (1927), and Bernstein (1928) believed *Caduceia* to be a synonym of *Devescovina*. Grassé probably has changed his opinion, since he recently (1937) used the name *Caduceia theobromae*. The structural differences are in degree rather than kind, as is also true in *Macrotrichomonas*. Nevertheless, there is evident a

marked distinction between this group of large flagellates and the very uniform group of *Devescovina*, and generic distinction is as justified, it seems, as between *Foaina* and *Metadevescovina*, or between *Devescovina* and *Macrotrichomonas*. In the interests of a useful taxonomic treatment of the Devescovinae, it is necessary to retain *Caduceia* as a separate genus.

Five species of *Caduceia* have been distinguished by the writer in material he has studied. Of these, only *C. nova* and *C. theobromae* have been described. The diagnosis just given is based on all of them, however. A sixth species is *Caduceia pruvoti*, a new combination for part of the species "*Devescovina pruvoti*" described by Duboseq and Grassé (1929). In that account they evidently confused species of *Tricercomitus*, *Foaina*, *Devescovina*, and *Caduceia* under the one species name.

All determined hosts of *Caduceia* belong to the genus *Neotermes*.

Caduceia theobromae França

(pl. 1, figs. 1-7; pl. 2, fig. 8; pl. 6, figs. 42-44; figs. A, B)

Caduceia theobromae França, 1918, Bull. Soc. Port. Sci. Nat., 8:94, pl. 2, figs. 1-8, figs. A, B, C3, D2 (type host—*Neotermes gestri* Silvestri. Island of St. Thomas).

Devescovina (Caduceia) theobromae França. Duboseq and Grassé, 1927, Arch. zoöl. exp. gén., 66:452.

Devescovina theobromae. Bernstein, 1928, Arch. f. Prot., 61:30.

Caduceia theobromae. Grassé, 1937, C. R. Soc. Biol., 125:918.

Hosts.—*Neotermes gestri* Silvestri. Island of St. Thomas.

Kaloterme (*sensu lato*) sp. East Africa.

T-2013. Mt. Meru. T.T. (Homosyntype slides TP-1083:6, 8.)

T-1063-1065. Moshi, Tanganyika Territory.

T-1092, 1094. Taveta Forest, Kenya.

T-3015. Near Rutchuru, Belgian Congo.

Diagnosis.—Length 71-124 μ , averaging 90 μ ; width 38-69 μ , averaging 48 μ ; three anterior flagella; trailing flagellum a slender cord, usually shorter than body; cresta 8-12 μ , averaging 9.5 μ in length; parabasal body generally spiraled in broad, loose, uneven coils, number of turns 2-5, averaging 3; chromatic shield of small, deeply staining granules applied to nuclear membrane; nucleus 15.5-18 μ \times 6-8 μ , averaging 17 \times 7.2 μ ; slender, rod-shaped micro-organisms adherent to a restricted area at the posterior end; slender spirochaetes, often 4-6 μ long, forming a dense coat over the rest of the surface.

That França's description is incorrect is evident to one who understands the comparative morphology of Devescovinae. Since comparison with type specimens has not been possible, identity of the flagellate from the Tanganyika termite with *C. theobromae* is uncertain, but the probability is very great. Certainly if any devescovinid is to be identified with França's description, it would be this one alone of all those known. With one unimportant exception his account might have been prepared from the flagellate studied by the writer.

França recorded observations of the living flagellates. Movements, he stated, are very rapid. Sometimes he observed the animal to be rounded with rotatory movements, sometimes elongated with forward movements. He also noted that the body is very plastic and may pass through a narrow space.

The writer studied living material in Tanganyika. The flagellate's activities are much like those of *Pseudodevescovina uniflagellata*. At the anterior end there is a small papilla and a group of three fine, relatively short, anterior

flagella. The papilla, with the group of anterior flagella, is continually in vigorous movement. Usually the bases of the flagella are either directed transversely, or approximately at a 45° angle anteriorly. The change in position of the papilla is brought about by lateral bending, which was always observed to be toward the left side—if the blepharoplast is considered to be ventral and the tip of the papilla dorsal. The trailing flagellum is relatively short and slender and its activity is comparatively feeble.

This flagellate, like *Pseudodevescovina uniflagellata*, is active in a way that seems difficult to explain on the basis of movements of the four flagella alone. The entire body may be rounded and rotate rapidly around a transverse axis, like *Trichomonas termopsisidis* (Kirby, 1931); or it may revolve more or less rapidly to the left around a longitudinal axis when abutting against débris as if trying to bore through. The cytosome is exceedingly labile. The anterior part of the body, with parabasal and axostyle, may revolve to the left while the part behind the parabasal is stationary; or the anterior part, with parabasal and axostyle, is stationary while the posterior part turns. Independent streaming and movements of the cytosome may occur, but there is no formation of pseudopodia.

Maintenance of the form of the papilla is aided by a lamella, which stains deeply with iron-haematoxylin, that extends from alongside the nuclear membrane and passes along its anterior margin. Probably this is part of the capitulum of the axostyle.

One flagellar root meets the blepharoplast directly; the other ends at a separate enlargement near the blepharoplast, attached to it by a slender fibril (pl. 1, fig. 5). In the size and shape of this enlargement there is much variation. Sometimes it is apparently only a thickening of the flagellar root; at the other extreme it is a granule as large as the blepharoplast. The granule sometimes is elongated in a direction at an angle to the line between the blepharoplast and the flagellar root.

The blepharoplast is a granule of constant size. In material sufficiently destained it is resolved into a number of small, deeply staining granules in a less stainable matrix—a structure shown more clearly in the larger blepharoplast of another species of *Caduceia* observed by the writer.

To the blepharoplast are directly attached: one anterior flagellum; the fibril from the granule at the end of the root of the other two anterior flagella; the trailing flagellum; the cresta; the parabasal filament, which runs close to the anteromedial edge of the cresta; a slender rhizoplast to the nuclear membrane; and a short rod, which is attached to its posterior part (pl. 1, fig. 5). This rod is constant in occurrence and position. The cresta is in both size and form much like that of *Devescovina lemniscata*.

One of the two large flagella that França described is directed posteriorly in several figures on his plate. What he figured is actually the trailing flagellum and cresta, the cresta being mistakenly regarded as a broadened base of the flagellum. It is obvious that this is so from the form and position of the supposed base, and the figures indicate that the cresta in França's material corresponded in size and form to that here studied. The other flagellum shown

by França is the group of three anterior flagella. For this he also shows a broadened base, mistaking for it the area enclosed by the two flagellar roots and probably also the anterior lamella.

The tubular extension to the nucleus (França, pl. 2, fig. 1; reproduced here as fig. A, 1) is the area bounded by the anteromedial edge of the cresta and the

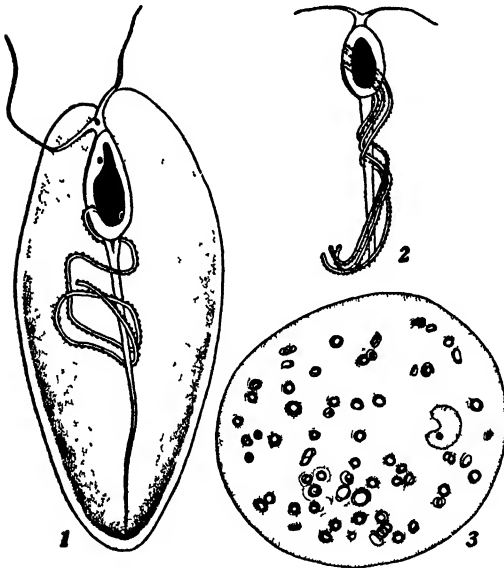


Fig. A. 1, 2. *Caducea theobromae* França. Copies of França's figures, plate 2, figs. 1 and 8. The adherent spirochaetes believed by França to be cilia are omitted. The direction of the spiral of the parabasal body is incorrect in both figures. The flagella and cresta are wrongly represented. The row of granules along the parabasal is doubtful; possibly França misunderstood the parabasal thread. No measurements or magnifications were given by França. 3. *Pseudodevescovina ramosa* n. sp. Spirochaetes in vacuoles (?) in cytoplasm. Shape of nucleus characteristic. Parabasal apparatus shown in specimen but is omitted from drawing. F. R. $\times 580$.

continuation of the anterior lamella to the nucleus. The granule figured between the two flagella probably is the blepharoplast or the granule at the end of one of the flagellar roots.

In its loose, irregular coiling the parabasal body is unlike that of most Devescovininae. Rarely it is tightly spiraled (pl. 1, fig. 6), only a few specimens out of thousands having it so. França showed the parabasal more slender than it is in the specimens observed by the writer, but he probably was inaccurate since the caliber of the parabasal does not differ so much in related species of Devescovininae. França's article includes eleven figures showing the parabasal body. Eight of these represent the spiral in a clockwise direction (fig. A, 1, 2), and three are counterclockwise. A clockwise spiral has not been observed by the writer in any one of thousands of individual devescovinids.

França stated that the parabasal seems to originate at a point on the nuclear membrane. Its main substance begins along the posterior part of the nucleus, but the parabasal thread extends obliquely along the nuclear membrane to the blepharoplast (pl. 1, figs. 4, 5). Following the short part applied to the nuclear membrane is a transverse loop with diameter equaling or, more frequently, exceeding the transverse diameter of the nucleus. Usually this is turned around the trunk of the axostyle just posterior to the nucleus. This loop appears in França's figures. The same type of loop and anterior part is characteristic of the parabasal of *Caducea nova*.

Beyond the loop the turns are wider, irregular, and variable. Successive parts are not often parallel to one another nor close together. The posterior loop generally is very wide, and sometimes it is turned forward over other parts of the spiral.

Because of the wideness of the spiral, the number of turns of the parabasal represents a greater length than it would in most other species of *Devescovininae* with coiled parabasal. This number ranges from 2 to 5, usually being about 3.

The parabasal body posterior to the nucleus is somewhat flattened, coming to an edge on the inner side where the parabasal thread is situated. In heavily stained material the entire parabasal is intensely black and no parts can be differentiated. When differentiation is sufficient for study of the cytology of the flagellate, the thread still stains intensely black, and is sharply marked off from the other substance, which stains more lightly gray or brownish (pl. 1, fig. 2). Even when the main substance is very pale, the thread still is black.

França did not show the parabasal thread as such. For the entire length of the parabasal body he represented along one border a row of granules (fig. A, 1, 2) which are regularly arranged and very close together. Dubosq and Grassé (1933, p. 511) suggested that França represented vesicles about to detach, presumably in the course of secretory activity of the parabasal. This is unlikely. No such granules have been seen by the writer along the parabasal body of any *devescovinid*, and it is possible that França saw, but misinterpreted, the thread. In most of his drawings, however, the granules are on the opposite side, and they are shown also in Delafield-stained material. Delafield's haematoxylin stains the entire parabasal deeply, but, as usual, the thread cannot be distinguished from the other substance, whatever the degree of destaining. The thread continuing to the blepharoplast is stained like the rest of the parabasal.

There are no blocks or granules like those in the parabasals of *Trichomonas termopsidis* and certain species of *Metadevescovina*, but clear vesicles, irregular in size, are present in the matrix (pl. 1, figs. 2, 4). All parabasals show these, generally in a single row, many being about a third the diameter of the parabasal body. Wavy unevenness in the outline of the parabasal is not pronounced, and there is no indication that the small vesicles have any relation to it.

In many specimens the parabasal body is constricted in one or two places, with the parts connected by a strand that probably is the parabasal thread (pl. 1, fig. 4). The position of this constriction varies.

Some flagellates have a part of the parabasal body separated from the rest and lying free in the cytoplasm (fig. B, 2-9). The place of separation varies. Usually a rather large spiraled part remains intact, and in some specimens the two parts added together would give an unusually long structure. Sometimes almost all, or rarely all, of the parabasal is so detached. Out of 300 specimens on one slide 20 had a detached part.

The two possible explanations of the separation are that it is caused by smearing, or that it happens normally in the course of the flagellate's life. The former explanation is unlikely, because neither the body nor either parabasal segment is distorted in the majority of separations. Also, the separated ends often lie far apart in a way that would require an appreciable shift in the position of the fragment, a shift that would differ greatly in different

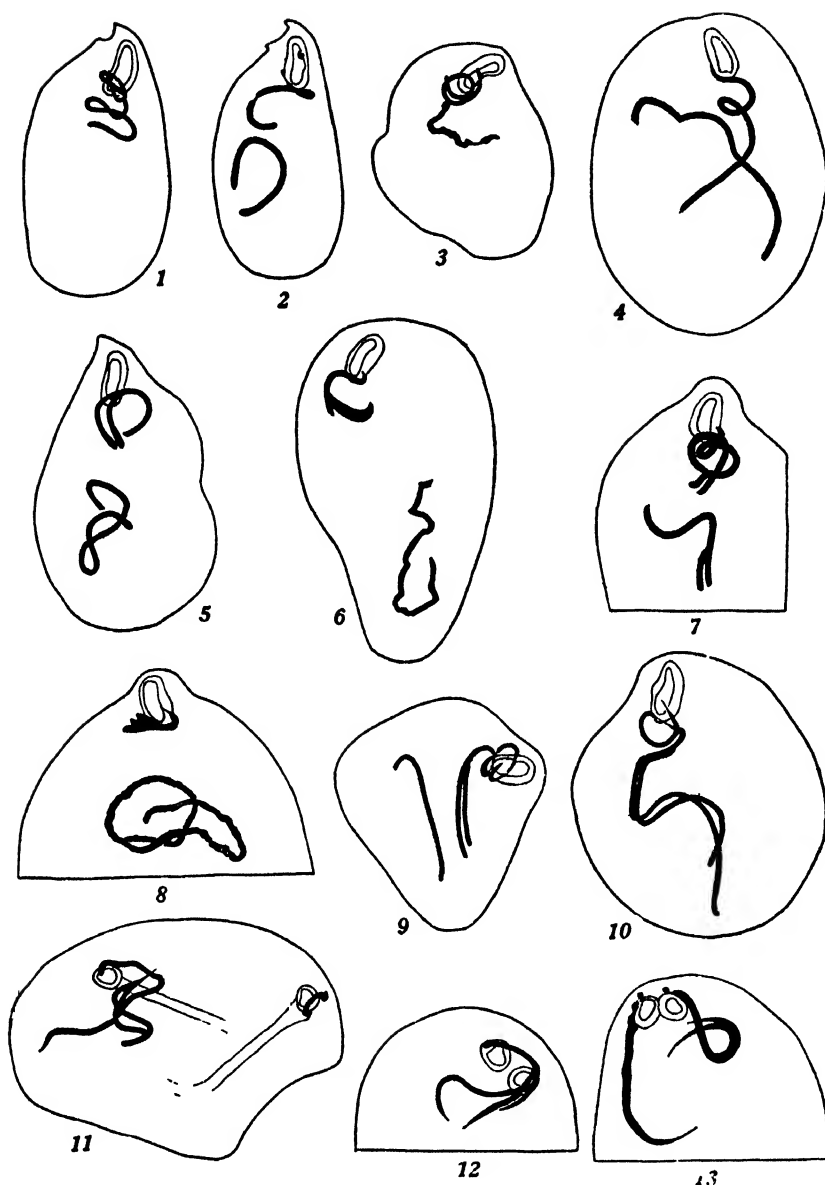


Fig. B. *Caduceia theobromae* França. Parabasal body. S. D. $\times 587$. 1. Normal, unbranched parabasal as in the great majority of specimens. 2-9. Part of parabasal detached, probably unrelated to division: 3. Detached part degenerating. 4. A long detached part probably once a branch of the other part. 5. Entire parabasal detached, in two parts, a branch on one part. 6. Two branches, in addition to the main part from which most of the parabasal has detached. 7. Detached and attached part, each with branch. 8. Attached part trifid, detached part degenerating. 9. Attached part with one branch and a region of separation of the main substance. 10. Unbroken parabasal, with a long branch. 11. Detachment of part of right-hand parabasal, and presence of a branch in late division stage. 12-13. Branched parabasal bodies in the telophase.

flagellates. Specimens so badly distorted in smearing that the cytoplasm was drawn out and occasionally the parabasal freed of cytoplasm, still had this structure intact.

The majority of the flagellates with detached parts of the parabasal lacked any indication of division, and occurred on slides without any division stages. When at the outset of division the parabasal is separated, a rapid disintegration of the detached part takes place. In detachment without division, however, very few of the fragments showed even slight evidence of degeneration. Although scores of them have been studied, the two figured (fig. B, 3, 8) were the only examples seen. Doubtless the fragment degenerates eventually, but the process is apparently slow. It seems probable that detachment of parts of the parabasal body occurs in *Caduceia theobromae* without relation to division. When such amputation occurs, the point of separation is variable. In the early prophase, separation is effected at a more constant place near the nucleus.

Branching of the parabasal occurs in a small percentage of flagellates (pl. 1, fig. 3; fig. B, 5-13). In 15 of 300 specimens on one slide there was branching. There is an outgrowth along the main limb, generally as stout as the rest, originating at any point posterior to the nucleus. Usually only the posterior part of the parabasal is bifid; sometimes the two parts are long and the branch begins near the origin of the parabasal. In two specimens there were three parts, two branches originating not far apart. These were short, however. No instance was seen of three parts equally long, such as França described (his pl. 2, fig. 8; reproduced here as fig. A, 2); but this would be but a step beyond some that were seen. França found two or three branches rarely. In three telophases, when the new parabasals were growing, a branch was present on one (fig. B, 11-13). The branch, like the main part, had the pointed end characteristic of developing parabasals.

The chromatic shield (pl. 1, figs. 2, 5) begins near the blepharoplast and extends down the nuclear membrane for half or two-thirds of the distance. It is a convex plate, thicker anteriorly where it extends away from the nucleus in a point, so that in section it has much the same form as a cresta. Its breadth is three or four microns. It is composed of numerous very small, closely packed granules.

The name chromatic shield is used for this group of granules because it is believed to be homologous with the structure described under that name by Connell (1932) in *Macrotrichomonas lighti*. It is present in all other species of *Caduceia* observed by the writer. It is probable that Duboscq and Grassé (1929) observed it in *C. pruvoti* but described it as the anterior part of the parabasal body, which they believed to consist of a fine, close spiral filament of about 25 turns, within which siderophile granules are enclosed. No parabasal body observed by the writer is anything like this, and the chromatic shield is the only structure known to the writer from which such a description might have been derived.

França's experiments with vital dyes led to no unusual results, but one which he used is of interest in connection with the homologies of the parabasal body. Having noted that oxazine has a special affinity for the "blepharoplast"

of trypanosomes, he attempted, without success, to stain the blepharoplast or parabasal body of *Caduceia*.

The cylindrical trunk of the axostyle is broad just posterior to the nucleus, and it contains the same type of elongated, siderophile cone that França represented. Posteriorly it tapers to a filament, as in *Devescovina*, with the end entirely enclosed in the cytoplasm. Usually, in fixed material, it is longer than the cytoplasmic body that must accommodate it, so that it is recurved (pl. 1, fig. 1).

Numerous rods similar to those reported in *Caduceia nova* (Kirby, 1936) are present in the cytoplasm (pl. 2, fig. 8). There is some variation in abundance, but on slides suitably stained to demonstrate them, after both Schaudinn's and Flemming's fluids, they are to be found in practically all individuals. They are somewhat stouter, average longer, and are more often curved or bent than are the rods adherent to the surface. The ends taper to points. Specimens measured range from 4.5 to 9 μ . They tend to aggregate in certain regions of the cytoplasm, particularly in the part adjacent to the area covered by the surface rods. As in *C. nova*, they are probably entozoic microorganisms.

The surface of the body bears microorganisms of two kinds. One is the *Fusiformis* type, very slender rods, pointed at the ends, many with a length of 3–4 μ . These are restricted to a circular area (pl. 1, fig. 7), generally on the posterior end, but sometimes, when the body is distorted, partly or entirely on one side. The area covered is comparable, relatively, in different specimens, and the boundary is very well defined. Within the area the rods adhere in groups, those in a group being parallel, the different groups being variously arranged.

Spirochaetes, many of which have a length of 4–6 μ , are abundant over the entire surface except the area occupied by the rods. França mistook these spiral organisms for cilia, and considered them to have long intracytoplasmic parts. He thought that along one border of the nucleus they arose from the semilunar plaque (chromatic shield) of numerous minute granules. This account of the intracytoplasmic part of the supposed cilia is certainly incorrect. França may have been misled by strands in the abnormal peripheral layer which, as his figures indicate, was present in his material; or by spirochaetes lying above or below the body. A tuft of longer spirochaetes is often adherent to the posterior end.

In what seem to be the best preserved specimens of *Caduceia*, *Devescovina*, and *Foaina*, from this termite as well as others, a clear zone between the granular cytoplasm and the surface layer of the body is narrow or absent. The cytoplasm containing granules, fragments of wood, and sometimes entozoic microorganisms extends almost, if not quite, to the periphery. Little alteration of this situation occurs in any smeared and fixed specimens of *Foaina*, but there is often an alteration in *Devescovina* and particularly in *Caduccia*. An outer clearer layer demarcated more or less sharply from the inner cytoplasm is differentiated. Its breadth varies in different individuals and in different parts of the same one; the poorer the general condition of the flagellate seems to be, the broader and more conspicuous it is. This outer layer is free of the

deeply staining granules and wood fragments of the endoplasm. In it are numerous small, palely staining granules and strands crossing in a direction in general vertical to the surface, though not uniformly so. In some specimens the strands are not evident, and the appearance is indistinctly finely alveolar. In others there are clearly defined strands and sometimes structures that look like tubules through the layer.

Formation of this layer is accompanied by swelling of the body; the broader the layer the greater is the indication of swelling. It may be the result of use of a hypotonic solution in dilution of the smears. The presence of rod-shaped microorganisms on the posterior area seems to impart to it a somewhat greater firmness than the rest of the body possesses. Swelling is less marked in this area. The difference is sometimes striking, bulging occurring just beyond the boundary of the "striated" area.

The material included a large number of division stages. As the process differs in no important way from that already illustrated in *devescovicinids*, few drawings are included in this paper. A complete account of the process will be included in a monograph on the group soon to be published by the writer. Much of the division material was stained in Delafield's haematoxylin, so that it showed well the reorganization of the parabasal body.

Many instances were seen of detachment of the posterior part of the parabasal. The detached part rapidly degenerates, breaking up into small, deeply staining fragments, not vesicles like those in *Pseudodevescovina uniflagellata* (Kirby, 1936). The proximal part, which remains attached, varies somewhat in length; generally it is about twice as long as the nucleus. Sometimes it is much shorter, but this is exceptional. Rarely no attached part is to be seen.

In many specimens in which the state of degeneration of the detached part indicated recent detachment, no second (new) parabasal body was to be seen (pl. 6, fig. 42). In others a slender filament, staining in Delafield's haematoxylin, was present at the other pole of the paradesmose (pl. 6, fig. 43). This filament reaches the length of the attached part before it thickens to full size, probably by accumulation of material from the surrounding cytoplasm. The persisting part of the old parabasal becomes pointed, and, like the new one, remains pointed during its growth (pl. 6, figs. 42-44).

Pseudodevescovina Sutherland

Pseudodevescovina Sutherland, 1933, Quart. Jour. Micr. Sci., 76:160; genotype *P. uniflagellata* Sutherland.

Diagnosis.—Three anterior flagella; one trailing flagellum, which is a relatively slender and short cord in the known species; cresta of small or moderate size; parabasal apparatus consists of a proximal element or main limb curving around the nucleus, or turned transversely beyond the nucleus, and a number of branches or cords attached to this; axostyle moderately stout, with pointed posterior end which may project from body; nucleus of the *Foaina-Metadevescovina* type; in known species, an investment of spirochaetes over almost the entire body; adherent rod-shaped microorganisms absent.

Pseudodevescovina is distinguished from other *Devescovininae* by the fact that the parabasal body has a number of branches or appendages on a transverse, uncoiled proximal element or main limb. The writer's study of the re-

production of the parabasal body in *P. uniflagellata* showed that these branches develop as outgrowths. A tendency to the development of such branches exists in other Devescovininae; it has been noted occasionally in several species of *Foaina*, *Metadevescovina*, and *Caduceia theobromae*, and is characteristic of the larger forms of *M. polyspira* and of *C. nova*. In *Pseudodevescovina* the possession of branches or appendages is a constant characteristic. *Parajoenia*, which has one parabasal appendage, is regarded as a separate genus mainly because of the peculiar form of its parabasal apparatus.

Pseudodevescovina ramosa new species

(pl. 2, figs. 9–13; pl. 3, figs. 14–16; pl. 6, fig. 45; fig. C)

Type host.—*Kalotermes (sensu lato)* sp., Tanganyika Territory. T-2012. Mt. Meru, near Usa. (Syntype slides TP-1085:13, 1082:4, 22.)

Diagnosis.—Length 40–113 μ , averaging 76 μ ; width 27–60 μ , averaging 42 μ ; flagella, especially the trailer, shorter than body, except in small individuals; trailing flagellum a relatively slender cord; cresta length 5.3–7 μ , averaging 6.5 μ ; parabasal apparatus composed of (1) a long main limb, which is a chromophile band and filament except in its proximal part, and which passes more or less transversely after bending in a U around one end of the nucleus, (2) a short curved branch arising near the bend of the U, and (3) a voluminous branched part that arises in 1–3 trunks close together near the bend of the U and subdivides at various points to produce 9–16 cords, which wind rather closely around the axostyle just posterior to the nucleus; trunk of axostyle stout, with a slightly enlarged cusp and a pointed projecting end; capitulum of axostyle considerably expanded; nucleus with no space between the membrane and the finely granular chromatin, with one or two peripheral nucleoli; nucleus usually elongated in an oblique axis, size 8.8–15 $\mu \times$ 4.4–8.8 μ , averaging 11.8 \times 6.6 μ ; characteristic elongated granules present just under surface over all the body; spirochaetes, many 7 or 8 μ long, abundant on entire surface.

When this large devescovinid was first seen, a close resemblance to *Pseudodevescovina uniflagellata* was immediately noted. Though the parabasal apparatus is different, in living material it seems similar, being divided into a number of cords. The body is broad, stout, and rounded at the ends. A relatively small papilla of *Devescovina* form is present at the anterior end.

The flagella, cresta, and parabasal body are attached to a relatively small, compact granule which does not appear to be made up of separate granules as has been observed in some other Devescovininae. Posterior to this granule is a larger, more deeply staining granule or pair of granules (pl. 2, figs. 9–11; fig. C, 1, 2). It is possible that when there appears to be only one posterior granule, the two are either pressed closely to each other or one lies over the other. There is some variation in the size of this granule or granules.

From this group of granules to the nuclear membrane there extends a well-defined, deeply staining rhizoplast, which has a sinuous curve and ends in contact with the nuclear membrane without a terminal granule or marked enlargement (fig. C, 1, 2). Proximally the rhizoplast passes the posterior granule or pair of granules at one side. The rhizoplast is a constant structure.

The extraordinary parabasal apparatus is composed of so many cords wound so closely around the axostyle just posterior to the nucleus that its structure is difficult to analyze. A large number of specimens stained after Schaudinn's fluid in Delafield's and Heidenhain's haematoxylin, and after Flemming's fluid in the latter, have been studied in preparing the following description.

The proximal element or main limb, which corresponds to the entire parabasal body of most devescovichids, is overshadowed by the large, ramified distal elements. As in many other devescovichids, the blepharoplast is not at the terminal point; there is a short hook with the blepharoplast at the bend (fig. C, 3). After passing posteriorly against the nucleus close to the cresta, the proximal element turns transversely and anteriorly in a broad curve, thus having a U shape. It then turns across the body, continuing to the opposite edge of the

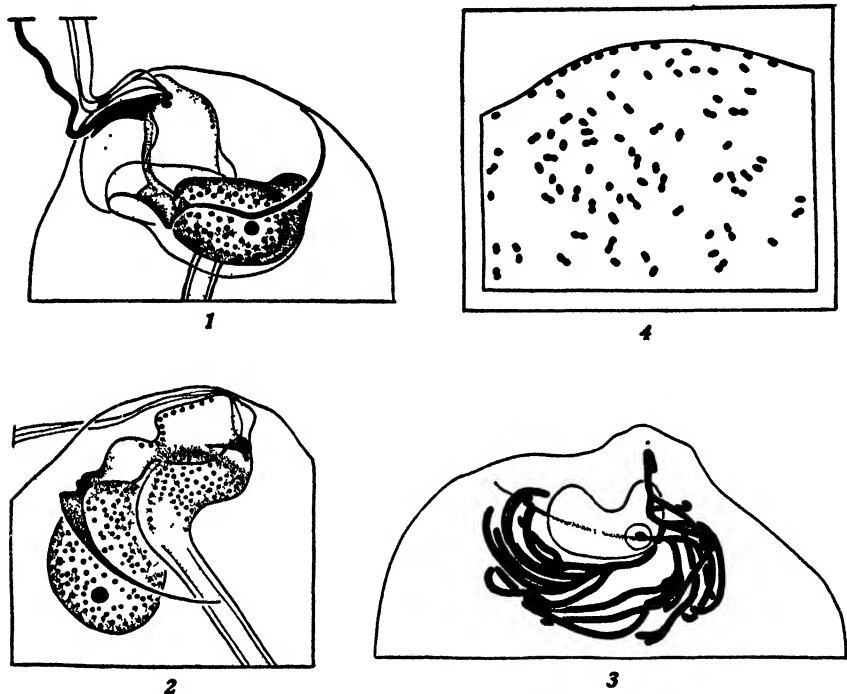


Fig. C. *Pseudodevescovina ramosa* n. sp. 1, 2, 4, $\times 1760$; 3, $\times 1280$. 1. Flagella, cresta, and proximal element of parabasal apparatus, showing attachment to granule; accessory granule. S. H. 2. Proximal element of parabasal, capitulum with uneven anterior border, capitular granules. F. R. 3. Parabasal apparatus, showing anterior hook and ramifications of distal elements. F. D. 4. Diagram of peripheral granules, showing apparent division. These granules are present beneath the surface layer of the entire body.

body in a somewhat variable direction. In general, it is more or less transverse and peripheral in position, at the level of the anterior end of the nucleus. Sometimes, instead of being peripheral, it passes across the nucleus against the nuclear membrane. When peripheral, it may pass transversely entirely anterior to the nucleus; or its terminal part may turn posteriorly; or, after passing along the nuclear membrane for a short distance, it may turn forward in a sharp curve (pl. 2, fig. 10).

The parabasal body, as usual, consists of two substances, one staining well with Heidenhain's haematoxylin after Schaudinn's fluid and the other not, but taking Delafield's haematoxylin well. In specimens prepared by the former method, there is next to the nucleus a deeply staining border along the edge of the part of the parabasal anterior to the bend of the U. Before reaching the bend this fades out. On the other, crestal, side a deeply staining border begins

about halfway to the bend and continues around it. Beyond the bend, the parabasal is a flat, deeply staining band that reaches a breadth of one micron, then tapers gradually to a stout terminal filament (pl. 2, fig. 11). The Delafield-staining substance, which constitutes almost all of the parabasal anterior to the bend, as well as the distal elements, is absent or scanty along this band and filament.

At the beginning of the bend there arises a comparatively short, curved cord, which passes around the small end of the nucleus (pl. 3, fig. 16; fig. C, 3). The curved end of this is situated just at the edge of an expansion of the capitulum.

The rest of the parabasal apparatus also is attached to the proximal element at or near the bend of the U (pl. 3, fig. 16; fig. C, 3). The cords, instead of originating separately along a considerable section of this limb, as in *Pseudo-devescovicha uniflagellata*, have their origin in one trunk, or more frequently two or three trunks very close together, with subsequent repeated branching. When there is a single trunk, there is almost immediate branching into two or three parts.

A cord that originates directly from the proximal element or from a trunk close to this may continue unbranched, or, more frequently, is branched repeatedly at various distances from its point of origin. All cords and branches are of the same caliber. Branching is doubtless accomplished by outgrowth.

In most specimens the writer was unable to count the number of ends, and in many of those in which an estimate was possible he could not be certain of the number. The counts made in 28 specimens were only approximately accurate, but the average number 12 is probably correct for the species. In these 28, the number ranged from 9 to 16; 9, 10, 15, and 16 being exceptional. These numbers do not include the short anterior cord that leads off laterally around the end of the nucleus.

In suitably prepared iron-haematoxylin material, there is a deeply staining border or filament along all the branches of the parabasal apparatus.

In many specimens the trunk of the axostyle has a diameter of about 2μ at the nucleus, decreasing to about 1μ near the posterior end. The terminal part is a typical cusp. It is first expanded a little, with the membrane heavier and more deeply staining than elsewhere; then it projects from the body, tapering at first abruptly, then gradually, for a distance of about 9μ in many specimens. The end is a slender filament. The trunk of the axostyle is composed, as are other large *devescovichid* axostyles, of a well-defined outer sheath and an inner core, with a clear space separating the two.

The trunk of the axostyle extends along one side of the nucleus without expanding greatly until it approaches the anterior end (pl. 2, fig. 10). The nucleus is elongated in a direction approximately transverse to the axostyle, and is concave on the axostylar side (pl. 2, figs. 9, 12). The axostyle thus is fitted into a groove in the nucleus.

There is an extensively expanded capitulum (fig. C, 1, 2). The nucleus is concave on the side toward the blepharoplast, and the greater part of the capitulum is on this side. The capitulum is first extended in a broad lobe toward

this granule, approaching the edge of the body so that the blepharoplast lies against the lobe. To the right of the blepharoplast as it is situated on the ventral surface of the body there is a rounded capitular lobe in which are some well-defined siderophile granules (pl. 2, figs. 10-12). Some similar granules are generally present also in other parts of the capitulum, but the group is most conspicuous in this lobe. The capitular expansion extends into the papilla, and then continues, with a broadly sinuous margin, on the left side of the nucleus (fig. C, 2).

The nucleus is similar to those of *Foaina*, *Metadevescovina*, and *Parajoenia*, and is markedly different from the nuclei of *Caduceia nova* and *Devescovina* sp. on the same slides. Its shape (fig. A, 3) resembles that of a segment of a thick doughnut, amounting to somewhat less than half, with the upper end of the trunk of the axostyle fitted into the concavity. The longer axis of the nucleus is oblique to the transverse axis of the body. The chromatin is abundant, comparatively finely granular and not heavily staining, and fills practically all the space under the membrane. Posteriorly the membrane is separated to some extent from the chromatin, and is extended into a ridge, an optical section of which appears as a narrow, toothlike projection (pl. 2, fig. 12). There are one or two deeply staining nucleoli.

In many specimens the texture of the cytoplasm is uniform to the periphery of the body, so that there is no well-marked distinction between ectoplasm and endoplasm. The food inclusions do not, however, occupy the peripheral cytoplasm. This peripheral region varies in width.

In other specimens, owing probably to unsatisfactory treatment before fixation, there is a well-differentiated outer layer, generally several microns in thickness, composed of cytoplasm that is finely alveolar and free of coarse granules or other inclusions. The granules which abound in the inner cytoplasm are limited sharply at the boundary. This layer seems to be increased in width by swelling, possibly as a result of immersion in hypotonic media.

The cytoplasm in flagellates from wood-fed termites contains numerous fragments of wood, more than are usually present in *Caduceia nova*. Besides these wood fragments, there are in many specimens a few to many spherules of variable size, which lie in clear zones; they probably are food vacuoles. These may be products of wood digestion. Granules are numerous; some are short rodlets which stain deeply with iron-haematoxylin and resemble entozoic bacteria.

Larger than these very abundant endoplasmic granules are the peripheral granules whose presence is very characteristic of this flagellate (pl. 2, fig. 13; fig. C, 4). They are present over the entire body, all situated just under the surface layer. Their distribution is uneven, and they seem clearly to be microorganisms, not structures of the flagellate. They can be stained deeply with either Heidenhain's or Delafield's haematoxylin after fixation in Flemming's or Schaudinn's fluids. Their form is that of short, blunt rods, variable in length; some elongated ones show a clear area in the middle and others show constriction apparently indicative of binary fission (fig. C, 4).

In specimens in which there is a clear outer layer, this layer often is crossed

by fibrils which are irregularly arranged and generally not straight and which seem to originate peripherally close to, but not in actual contact with, the peripheral granules. It is probable that these fibrils are artifacts, as they do not appear in what seem to be the best fixed specimens. Similar apparent fibrils crossing the outer layer occur in other flagellates, as in *Caduceia nova* and *Devescovina* sp. on the same slides from this termite. Those flagellates have no peripheral granules.

Many specimens, especially those from termites fed for several days on filter paper, contain spirochaetes in the cytoplasm. Some of these have a normal spirochaete form, but most of them are rolled up in vacuoles (fig. A, 3). Of fifty specimens examined in succession on one slide from a termite that had been four and one-half days on filter paper, the rolled-up spirochaetes were present in thirty, and were very abundant in seventeen. Most of them, even when rolled up, appeared intact. Among them were some rounded bodies, and an occasional disintegrated spirochaete, but there was evidently not much digestion in progress. A few spirochaetes were present in specimens of *Pseudodevescovina ramosa* from wood-fed termites, but they were much less frequent than in the previously described material. The surface of the body bears a coat of rather slender spirochaetes, many of which are 7 or 8 μ long (pl. 2, fig. 13).

Very few division figures of *Pseudodevescovina ramosa* have been found. A careful search was made for them in the hope that light would be thrown on the origin of the parabasal apparatus. The binucleate stages observed were not so valuable for this purpose as prophases would have been, but they did indicate some significant things. The proximal element of the parabasal apparatus was in these post-telophase stages a long, slender, somewhat crooked filament, not broadened in any portion as in interphase specimens (pl. 6, fig. 45). Alongside the proximal part of the filament was some chromophobe material, but this was absent along its distal part. Some poorly stainable cords were visible, attached to each of the two filaments, near the nuclei. Evidently the ramified part of the apparatus had already started to develop but had not advanced to the size characteristic of interphase specimens. The branched part probably buds out from the main limb, as do the cords of *Pseudodevescovina uniflagellata*, but in *P. ramosa* the outgrowth takes place much sooner.

The paradesmose is a stout rod-formed structure, which persists as a strand along the axostyle in postdivision specimens that have not yet completely reorganized.

Macrotrichomonas Grassi

Macrotrichomonas Grassi, 1917, Mem. R. Accad. Lincei, (5) 12:376; genotype *M. pulchra* Grassi.

Gigantomonas Connell, 1932, in part, Univ. Calif. Publ. Zool., 37:180 (not *Gigantomonas* Dogiel).

Diagnosis.—Devescovinids of large size, known species 36–91 μ long, averaging 52–68 μ ; distance from anterior end to nucleus varies, of value for taxonomic purposes; three anterior flagella; trailing flagellum well developed, broad band-formed in some, usually about 1½ times length of body; cresta developed into a broad internal membrane, 25–49 μ long or more; parabasal body usually unbranched, tightly coiled around axostyle 1–13 times; capitulum moderately developed; trunk of axostyle stout, tapering, but not reduced to fila-

ment as in *Devescovina* and *Caduceia*, no terminal enlargement, and generally pointed, usually projecting a short distance; nucleus of *Devescovina* type; no longitudinally adherent rod-shaped microorganisms; long spirochaetes present on anterior and posterior parts of some species.

The diagnosis just given, like that of *Caduceia*, is based on several undescribed as well as the described species of *Macrotrichomonas*.

In a postscript to the paper in which he described *Macrotrichomonas pulchra*, Grassi stated his opinion, based only on a review of Dogiel's paper, that his genus name should be a synonym of *Gigantomonas*. Connell (1932) concurred in this opinion. It is probable, however, that the genera are not synonymous. The writer is convinced of this after comparison of stained smears and living material of *Gigantomonas herculea* from *Hodotermes mossambicus* with all known and several undescribed species of *Macrotrichomonas*.

Macrotrichomonas pulchra Grassi

(pl. 3, figs. 17-19; pl. 4, figs. 20-27; pl. 5, figs. 28-38; pl. 6, figs. 39-41)

Macrotrichomonas pulchra Grassi, 1917, Mem. R. Accad. Lincei, (5) 12:50, pl. 9, figs. 1-12 (type host—*Glyptotermes parvulus* Sjöstedt, Gold Coast).

Gigantomonas pulchra (Grassi). Connell, 1932, Univ. Calif. Publ. Zoöl., 37:180.

Gigantomonas pulchra. Grassé, 1937, C. R. Soc. Biol., 125:918.

Hosts.—*Glyptotermes parvulus* Sjöstedt. Gold Coast.

T-301 (Silvestri). (Neosyntype slides TP-318:1, 2.)

Kalotermes contracticornis Snyder. Costa Rica.

T-132. Cartago. (Homosyntype slides TP-72:15, 12, 14, 19.)

Glyptotermes montanus Kemner. Java.

T-324 (Cleveland-Collier). (Homosyntype slide TP-262:11.)

Glyptotermes ceylonicus Holmgren. Ceylon.

T-314 (Cleveland-Collier). (Homosyntype slide TP-265:5.)

Diagnosis.—Dimensions of body: from *G. parvulus*, length 44-91 μ , averaging 67 μ , width 21-41 μ , averaging 32 μ ; from *K. contracticornis*, length 42-82 μ , averaging 57 μ , width 16-33 μ , averaging 25 μ ; from *G. montanus*, length 36-68 μ , averaging 49 μ , width 16-31 μ , averaging 23 μ ; from *G. ceylonicus*, length 37-66 μ , averaging 52 μ , width 19-39 μ , averaging 26 μ , distance from anterior end to nucleus 5-9.5 μ , usually 7-8 μ ; trailing flagellum a broad band, maximum about 1.8 μ , part proximal to body surface more deeply staining; length of cresta 25-35 μ , anteromedial edge about 14-18 μ , posteromedial edge about 17-26 μ , maximum width about 7-11 μ , anteromedial edge bordered by a paracrestal filament which continues posteriorly inside the coils of the parabasal body; no marked thickenings of cresta; parabasal body coiled tightly around axostyle 2-13 turns, usually 3-4; anterior lamella, suspensory lamella, and well-defined bent rhizoplast present; nucleus, from *G. parvulus*, 9.2-10.7 \times 6.8-9 μ , averaging 9.6 \times 7.7 μ , from *K. contracticornis*, 7.5-10 \times 5-7.5 μ , averaging 8.3 \times 6.4 μ ; clear zone under membrane frequently evident; spirochaetes 15-20 μ long sometimes present on anterior and posterior ends.

It may be surprising that the same species occurs in the African, Central American, and Javan termites, but a careful comparison shows no differences except in size. As only one slide from *Glyptotermes parvulus* could be studied, and the situation in other devescovinids indicates little significance for small discrepancies in general size, this character cannot be used for taxonomic separation.

Grassi's only figure of an entire *Macrotrichomonas pulchra* represents an animal 57 \times 35 μ , but it is a somewhat rounded-out specimen. In normal living

material, the flagellate is not expanded and broadly rounded in its posterior portion, as in Grassi's figures, but many specimens on smears have this shape.

The three long anterior flagella pass along the anterior border of the small papilla in two roots, the anterior of which is stouter than the other. At the proximal end of these roots (pl. 3, fig. 19; pl. 4, figs. 24-26), close to the anterior end of the body at the base of the papilla, are the two granules described by Grassi. The anterior of these appears as simply the enlarged end of the anterior root, while the posterior one, which is similar in size, appears as a more definite granule because of the smaller caliber of the root.

Posterior and close to these two granules are two or three others, all together forming a rough square (pl. 4, figs. 23-25). At one posterior corner is a small granule that is met by the cresta and trailing flagellum; at the other is a larger granule to which the rhizoplast and parabasal are connected; and within the square is a minute granule that apparently is joined by the paracrestal filament described below.

Grassi describes a delicate filament connecting the two granules at the ends of the anterior flagella, and thinks probable the existence of a filament between one of them and the granule at the end of the "undulating membrane" (trailing flagellum). It is very difficult to see these connections, and the writer has not been able to observe one between the granules of the anterior flagella. There do seem to be interconnecting fibrils between the anterior flagellar granules and the minute central granule, and between that granule and the two posterior granules (pl. 4, figs. 24, 25).

The trailing flagellum is a slender filament at the point where it leaves the granule, but it soon broadens to a band. It is at maximum breadth of about 1.8μ for almost all the distance along the cresta, differing therein from the usual situation in *Foaina*, *Metadevescovina*, and *Devescovina*. Posteriorly it tapers to a filament. Though ribbon-formed, it is not so simple a band as in certain species of *Devescovina*. The side that normally is adjacent to the surface of the body stains deeply with iron-haematoxylin to a variable breadth of about a third or half of the flagellum (pl. 4, fig. 24). The rest of the flagellum is a thin, clear membrane bordered by a stainable filamentlike edge. This edge is somewhat longer than the other, so that the flagellum is rippled. In the posterior part the relative width of the clear membranous region decreases, and the terminal part of the trailer is a continuation of the deeply staining substance. The resemblance to an undulating membrane with costa and axoneme is striking, but it is merely a superficial resemblance. The costa of a true undulating membrane is intracytoplasmic and would not normally be detached. Furthermore, a study of the comparative morphology of the Devescovininae indicates that it is the cresta that is comparable to the costa.

The relation of the trailing flagellum to the surface of the body has not been studied in living material. On well-preserved smears, fixed in Schaudinn's fluid, it is clear that there is no *fusion* of the flagellum to the edge of the cresta or the surface of the body. The flagellum usually, however, parallels the edge of the cresta, following its undulations (pl. 4, fig. 21) and correspondingly changing its direction when, as in many fixed specimens, the cresta is wound

the beginning of the coiled part (pl. 4, fig. 27). The branch usually was much shorter than the main limb; when sufficiently long, the branch was coiled between the turns of the main limb.

The nucleus resembles that of *Devescovina* in its longitudinal position, and in the frequent presence in Schaudinn-fixed material of a clear zone between the central chromatin mass and the well-defined membrane. In the central chromatin mass there are numerous granules of variable size, some large, and a large nucleolus is present (pl. 4, fig. 22). The chromatin mass often is pointed posteriorly, but there is no prominent posterior projection of the nucleus. On the slide from *Glyptotermes parvulus* the space under the membrane was very broad and the chromatin mass small (pl. 3, fig. 19), evidently a result of unusual shrinkage from fixation.

The endoplasm is crowded with inclusions. Occasionally in otherwise normal nondividing stages a small part of the posterior end of the parabasal has been detached (pl. 5, fig. 29). The detached part may be close to the normal coiled part, and likewise coiled around the axostyle, or may be free in the cytoplasm. This apparently has nothing to do with the division process; it suggests that during normal vegetative life the parabasal may increase in length, and that pieces may at times be separated and resorbed in the cytoplasm.

Nutrition was normal in the flagellates studied, as the smears were made soon after collecting the termites from their native habitat. In addition to abundant granules, there are many wood fragments, mostly small, but some relatively large. In many specimens vacuoles containing other protozoa have been observed. Most frequent in these is a species of *Foaina*, of which there were sometimes a number in one large vacuole. Another species of *Foaina* and *Trichomonas cartagoensis* have also been found present, usually occurring singly in vacuoles.

Peripherally there is a narrow zone of ectoplasm, granular but without other inclusions. In many specimens, however, this is abnormally broad. The cytoplasm of *Trichonympha chattoni* on the same slides shows no such alteration, so apparently it is due to some abnormality of treatment to which *Macrotrichomonas* is especially susceptible, like *Caduceia theobromae* and *Pseudodevescovina ramosa*.

On the bodies of most specimens from *Kalotermes contracticornis* a tuft of spirochaetes 15–20 μ long adheres in the region of the papilla. Sometimes a few are present also at the posterior end (pl. 4, fig. 20). On *M. pulchra* from *Glyptotermes parvulus* fewer spirochaetes were observed, though some were present. They were likewise less frequently present on *M. pulchra* from *Glyptotermes montanus*.

In each of three large specimens a smaller *Macrotrichomonas pulchra* was enclosed. Intracytoplasmic spirochaetes were of rather frequent occurrence. Within the cytoplasm they stained more intensely with iron-haematoxylin than those outside. Some were of normal form, others coiled or twisted irregularly. Occasionally spirochaetes were coiled up in vacuoles, but usually they lay directly in the cytoplasm. Many of them, at least, were probably alive before fixation.

The specimens of *M. pulchra* from *Glyptotermes ceylonicus* (pl. 3, fig. 17) showed some differences from those of *Kalotermes contracticornis*; probably they resulted from the technique of preparation. The paracrestal filament was not observed and the trailing flagellum appeared to be a somewhat narrower and more uniform band, though occasionally showing a darker border. In the material prepared by the Schaudinn iron-haematoxylin technique the trunk of the axostyle stained intensely black in some specimens and not in others on the same slide. Along the basal region of the trailing flagellum, between this and the body, there was often an irregular aggregation of black material, doubtless an artifact due to accumulation of stain. The shape of the body was usually abnormal, and the trailing flagellum was not parallel to the crestal edge. In an unusually large proportion of the material it was partly or entirely enclosed in the cytoplasm; sometimes the anterior part of the flagellum was free and the posterior part enclosed, or only the posterior part was free, or all was enclosed. It is clear from the foregoing that although the same chemicals were used, the results of fixation and staining were abnormal in the material; hence no stress can be laid on slight apparent differences. In some poorly prepared material from *K. contracticornis* the flagellum also appeared as a more or less uniformly staining band.

At the outset of division in *Macrotrichomonas pulchra* the body becomes rounded and the nucleus comes to be situated close to the anterior extremity. A stout paradesmose, with truncate ends, develops against the anterior surface of the nuclear membrane (pl. 5, figs. 37–38). The several separate granules to which various organelles are attached in the resting stage are replaced by a single minute granule close to each end of the paradesmose and attached to it by a fine filament (pl. 5, fig. 36). To this granule flagella and crestas, at least, connect. The result of fusion of the previous granules would be much larger in size than it is, so it is clear that most of the granule substance disappears. Especially noteworthy is the contrast between the stout ends of the roots of the anterior flagella of the resting stage, and the fine flagella emerging from this minute granule in division. The writer has no evidence concerning the relation of the earlier granules to the origin of the paradesmose in this flagellate.

The anterior flagella are distributed as in other devescovichids, two and one (pl. 5, fig. 35), and new ones early grow out to complete the number. The trailing flagellum persists, and from the granule at the other pole of the paradesmose a new one quickly develops (pl. 6, fig. 39). In the prophase it becomes longer than an anterior flagellum, and during the anaphase it becomes a narrow band. It does not equal the old one in breadth until after plasmotomy.

The paradesmose does not stain in Delafield's haematoxylin, and when heavily stained in iron-haematoxylin it seems to be a homogeneous black bar. Upon further differentiation in iron alum it no longer appears homogeneous. There are at least two well-defined fibrils, one stouter than the other, extending for the length of the paradesmose, each enlarged at the ends (pl. 5, fig. 37). The ends of the paradesmose retain the stain more firmly than the rest (pl. 5, fig. 38).

In the late prophase the paradesmose becomes depressed in a shallow groove of the anterior nuclear membrane (pl. 6, fig. 40). This is much less deep than the grooves in the nuclei of *Devescovina lemniscata* and *Devescovina cometoides*.

The old cresta soon becomes detached (pl. 6, fig. 39), crumples (pl. 5, fig. 36), and degenerates, but the paracrestal filament remains intact with its stouter distal portion. The new cresta develops beside it. At the other pole a new paracrestal filament and cresta develop in the prophase, the former more rapidly than the latter, soon reaching its full length (pl. 6, fig. 40). The distal part does not, however, enlarge fully until later. After the telophase the crestas reach full size (pl. 6, fig. 41). During early reorganization stages it is clear that the paracrestal filament is not a fused part of the cresta, as it separates from the anteromedial border (pl. 5, fig. 35).

Observations show that the axostyle behaves as usual in *devescovinids*, but the material was not favorable for studying this. Resorption of the old axostyle and differentiation of two new ones is probably universal in trichomonad flagellates.

At the beginning of mitosis the chromatin mass may be displaced toward the side where the paradesmose is situated (pl. 5, fig. 37). The larger chromatic bodies break up, and the granules aggregate into a number of irregular, elongate structures (pl. 5, figs. 38, 33). They have rather imperfectly defined outlines, and are granular, but they resemble chromosomes. The number appears to be constant; there are more than ten and probably not more than fifteen. In the telophase these aggregates break up again into scattered granules, which fill out the intranuclear region.

More than 150 division stages stained in Delafield's haematoxylin to show reorganization of the parabasal apparatus have been observed; of these, half have been carefully studied in composing the following description of that process.

In early stages the nucleus has migrated anteriorly so that the anterior end of the parabasal body lies close to the membrane (pl. 5, fig. 28). The capitulum of the axostyle probably has already been dedifferentiated, at least to some extent. While the old cresta is still unmodified and in place, and the nucleus shows little or no change, and probably before the paradesmose appears, there is a break in the parabasal at the beginning of the coiled part (pl. 5, fig. 28). The part remaining attached is about the length of the anteromedial edge of the cresta. The detached part at first remains coiled around the axostyle, but may be shifted posteriorly along the trunk. It then uncoils, at the anterior or both ends, and may or may not become free from the axostyle. If free in the cytoplasm, it becomes coiled, tangled, and separated into pieces. Whichever it does, by the late prophase it has been entirely resorbed (pl. 5, figs. 30-32).

Meanwhile the paradesmose has developed, with the persistent part of the old parabasal body at one pole. The old cresta is detached and is resorbed along with the greater part of the parabasal body (pl. 6, fig. 39; pl. 5, fig. 36). At the other end of the paradesmose, which as yet has not quite reached the diameter of the nucleus, a new parabasal body appears (pl. 5, figs. 30-31). This is at first

short, slender, and stains more lightly than the old one. Soon its anterior end becomes as stout as the other, but its posterior end is attenuated and pointed. Its length becomes equal to the other, which meanwhile usually has also become pointed. Both then increase equally in length, and by the late anaphase or telophase have almost, if not quite, attained the maximum (pl. 5, fig. 33). The ends are, in late stages, always rounded. When the nuclei are at opposite ends of the body, the parabasals are long, sinuous (pl. 5, fig. 34), and may be loosely and irregularly turned around the new axostyles. The tight coil of the typical nondividing individual is not developed until after plasmotomy.

DISCUSSION

It is beyond the scope of this paper to discuss at length the applicability to devescovinid flagellates of the modifications of the usual usage of the terms blepharoplast, rhizoplast, centriole, and paradesmose, that Cleveland (1934) has found necessary in hypermastigote flagellates. The observations on the division process reported here are but a small part of those made on Devescovininae, which are, however, not yet complete enough to warrant a general discussion. It is probable that the situation in these and other polymastigote flagellates will, when more complete data are available, be found to be comparable with that in hypermastigotes. A few facts bearing on the problem may be commented on here.

In division stages of various devescovinids there has been noted near each end of the paradesmose a small granule to which the flagella and certain other organelles connect. Between each granule and the corresponding end of the paradesmose is a short, fine fibril. These structures were reported in *Pseudodevescovina uniflagellata* (Kirby, 1936), *Parajoenia grassii* (Kirby, 1937), and *Macrotrichomonas pulchra* (this paper). They have been observed in other species.

What are the proper terms to apply to these structures? The writer referred to the granules as centrioles in *Pseudodevescovina*, and used the term blepharoplast for them in *Parajoenia*; further comparative studies are necessary before the confusion will be cleared up.

The filaments connecting the corresponding granules in *Trichomonas termopsidis* and *T. termitis* to the ends of the paradesmose are appreciably longer. Cleveland (1934) refers to them as elongate centrioles in the latter species, and shows them (pl. 60, fig. 41) as deeply stained, rather thick structures. The granule at the upper end he considers to be no more than the proximal end of the centriole. In the devescovinids the filament is very short and delicate, and the granule contrasts markedly with it in size.

If a centriole exists in the division figure, it is certainly to be sought in these structures. By comparison with Cleveland's observations, the granule with the filament would represent it; or the granule might be a centriole, with a filament connecting it to the paradesmose.

In the nondividing state, what structures correspond to these? The persistence of an elongate centriole in various nondividing hypermastigotes and in *Trichomonas termitis* has been described by Cleveland. In *T. termitis* the

structure so named corresponds to the filament that Kofoed and Swezy (1919), Andrews (1925), and Kirby (1931) called the rhizoplast in *T. termopsidis*, which is a very similar flagellate. A structure corresponding to this so-called rhizoplast has been described in many flagellates, and is present in devescovinids. It is a long, bent filament, for example, in *Macrotrichomonas pulchra* (pl. 4, figs. 24, 25). In *M. pulchra*, however, as well as in certain other devescovinids, it does not correspond in size and form to any structure observed to be associated with the division figure. The writer has used the term rhizoplast for it, because this corresponds to the structure so named in general usage, and there is no evidence at present concerning its relation to any structure in the division figure.

The arrangement of granules at the origin of the flagella, cresta, parabasal body, and rhizoplast differs in the three species described in this paper. The term blepharoplast has been used for the granule in *Caduceia theobromae* to which the various organelles connect, but in *M. pulchra* it is not clear which one, if any, should be so named, or whether all the granules should be called blepharoplasts. To clear up the nomenclature, a detailed review of usage and further study of cytological details is necessary. Cleveland (1934) discusses some of the difficulties. At any rate, the granule near the end of the parademesomere is not the equivalent of the several granules at the origin of the organelles of the nondividing cell. Much of the granule substance is absorbed at the beginning of mitotic changes.

The short rod attached to the granule that corresponds to a blepharoplast in *Caduceia theobromae* is not unusual in devescovinids. In the several species in which it has been found it is a constant structure with little variation in position or length among individuals of a species. It evidently is not undergoing growth. Comparison with Cleveland's observations suggests the possibility that it may have some relation to the later division figure, being a developing new centriole, which he finds in nondividing stages of hypermastigotes and *Trichomonas*. There is no evidence for that, however, in the observations on devescovinids so far made.

Grassé's statement (1937) that the "centrosome" engenders directly only the parabasal filament, and that the main parabasal substance differentiates in its vicinity rather than being a direct product, is borne out by observations the writer has made. In some species, in fact, the filament seems to parallel the other parabasal substance without direct contact with it (pl. 5, fig. 31).

Separation and resorption of part of the parabasal body apparently occurs normally, at the beginning of division, in at least many devescovinids. In some species, as in *Caduceia theobromae* and *Macrotrichomonas pulchra*, a similar process occurs also without relation to division. There is in this some evidence that the parabasal body is continually growing and separating parts, but not expelling vesicles as Duboscq and Grassé contend. In many species, however, there is no evidence of separation of parts without relation to division. In the elaboration and periodic resorption of this parabasal substance there is doubtless some physiological significance; but concerning what it is, one can only hazard guesses.

SUMMARY

1. The original account of the type species of *Caduceia*, *C. theobromae*, is incorrect. A flagellate that probably is identical with it has been studied and described in the light of present knowledge of the comparative morphology of the devescovinid flagellates.

2. Grassi's description of *Macrotrichomonas pulchra* is supplemented and corrected.

3. A second species of *Pseudodevescovina*, *P. ramosa*, is described from an African termite.

4. In its loose, irregular coiling the parabasal body of *Caduceia theobromae* is unlike that of most Devescovininae, including several undescribed species of *Caduceia*. It is similar to the main limb of the parabasal apparatus of *C. nova*.

5. Slender rod-shaped microorganisms are restricted to a circular area, generally on the posterior end of *C. theobromae*, as on *C. nova*.

6. *Pseudodevescovina ramosa* has a complex parabasal apparatus, comprising a long main limb that passes more or less transversely after bending in a U-form around one end of the nucleus, a short curved branch arising near the bend of the U, and a voluminous branched part that arises in 1-3 trunks close together near the bend of the U and subdivides at various points to produce 9-16 cords. The apparatus is curved rather closely around the trunk of the axostyle just posterior to the nucleus.

7. Elongated peripheral granules are present just under the surface layer of the whole body of *P. ramosa*.

8. In *Macrotrichomonas pulchra* the cresta has become a large internal membrane and the ribbon-formed trailing flagellum is weakly adherent to its external edge, but there is no undulating membrane like that of *Trichomonas*. Along the anteromedial edge, and extending beyond it posteriorly, is the paracrestal filament.

9. In division of *M. pulchra*, the several granules at the ends of the mastigont structures are replaced by a single small granule attached by a short filament to the end of the paradesmose. When well differentiated, the paradesmose shows at least two fibrils. The flagella persist, and new ones soon grow out. The cresta is detached and resorbed and two new ones develop, but the old paracrestal filament persists. The old axostyle is resorbed.

10. In *Caduceia theobromae* and *M. pulchra* the posterior part of the parabasal body is detached at the beginning of division, leaving a proximal part often about twice as long as the nucleus. The detached part quickly disintegrates. A new parabasal, at first filamentous, develops at the other pole of the paradesmose; it first becomes as long, and later as thick, as the attached part; then both grow equally until full size is attained.

11. In *C. theobromae* and *M. pulchra* separation of a posterior part of the parabasal body occurs in many specimens without relation to division. In *C. theobromae* the detached part remains intact in the cytoplasm longer than it does in division.

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EXPLANATION OF PLATES

All figures drawn with the aid of the camera lucida. Abbreviations: B., Bouin's fluid; D., Delafield's haematoxylin; F., acid fuchsin; FL., Flemming's fluid; R., Regaud's haematoxylin; S., Schaudinn's fluid.

PLATE 1

Caduceia theobromae França

Fig. 1. General: axostyle completely enclosed in cytoplasm, as is characteristic of the genus; spirochaetes that adhere to most of the surface omitted; the form and position of the anterior loop of the parabasal is characteristic of the species. S. D. $\times 1110$.

Fig. 2. Detail of the parabasal body, showing the deeply stained filament and poorly defined vesicles in the more lightly staining substance; upper end of chromatic shield anterior to nucleus. Fl. H. $\times 1330$.

Fig. 3. Parabasal with a branch on its posterior part. S. D. $\times 1330$.

Fig. 4. Showing how, as is characteristic of the species, the main substance of the parabasal body ends at the posterior part of the nucleus and the filament extends to the blepharoplast; two breaks in the substance of the parabasal. S. D. $\times 1330$.

Fig. 5. Semidiagrammatic; anterior part of parabasal, showing the characteristic form of the anterior part and the first loop; cresta; optical section of chromatic shield; roots of flagella; blepharoplast and flagellar granule, with interconnecting filament; short rod extending dorsally from the posterior part of the blepharoplast. Fl. R. $\times 1830$.

Fig. 6. An unusually close spiral of the parabasal body. The anterior loop, however, retains its characteristic form. Fl. H. $\times 1830$.

Fig. 7. Circular area at the posterior end with adherent rod-shaped microorganisms. Fl. H. $\times 1110$.

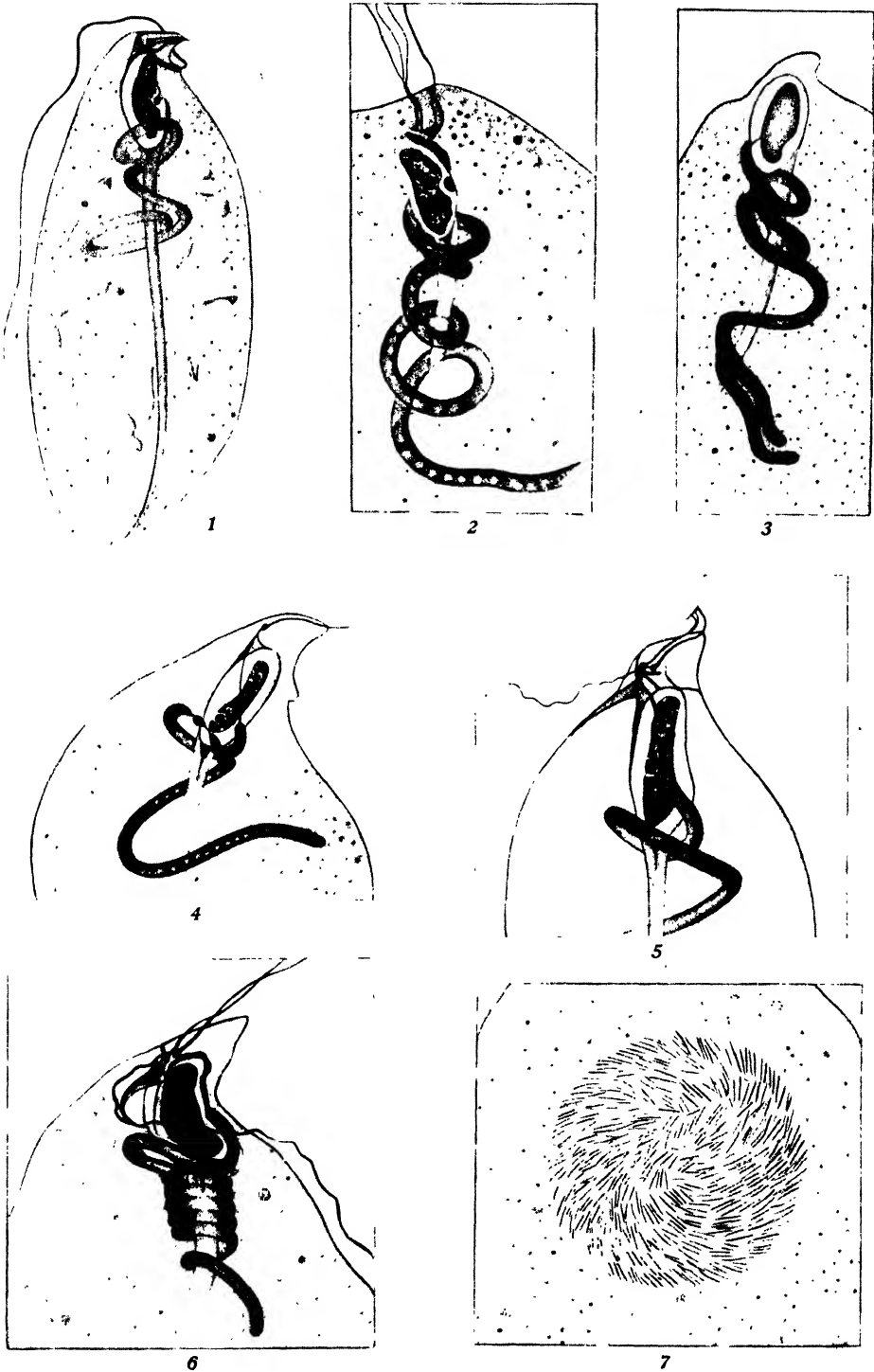


PLATE 2

Fig. 8. *Caduceia theobromae* França. Rods, probably entozoic microorganisms, in the cytoplasm. Fl. H. $\times 1110$.

Figs. 9-13. *Pseudodevescovina ramosa* new species

Fig. 9. General structure: trunk of axostyle and cusp; oblique position of nucleus; cresta and flagella; peripheral granules at margin. S. H. $\times 880$.

Fig. 10. Cresta; anterior end of trunk of axostyle and curvature of capitulum; sharp curvature of proximal element of the parabasal apparatus; nucleus with no space under membrane, fine chromatin granules and nucleolus. Fl. R. $\times 1830$.

Fig. 11. Cresta; blepharoplast and nearby granule; origin of the flagella and other structures of the mastigont; parabasal body, with one end of the proximal element at the blepharoplast and the posterior part over the nucleus as a deeply staining, tapering band passing toward the periphery of the body, and the numerous distal cords. Fl. R. $\times 1830$.

Fig. 12. Anterior end of trunk of axostyle, situated in a groove in the nucleus; part of capitulum with capitular granules; deeply staining terminal part of the proximal element of the parabasal apparatus; subdivided distal elements of the parabasal apparatus; blepharoplast with attached flagella and associated granule. Fl. R. $\times 1330$.

Fig. 13. External aspect: flagella, peripheral granules, and adherent spirochaetes. S. H. $\times 880$.

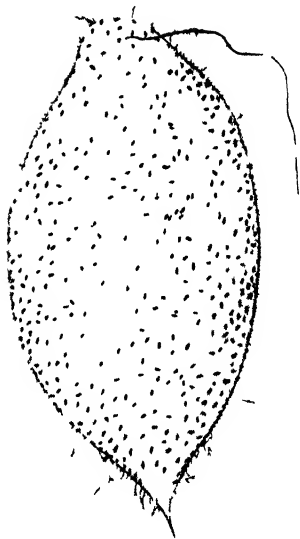
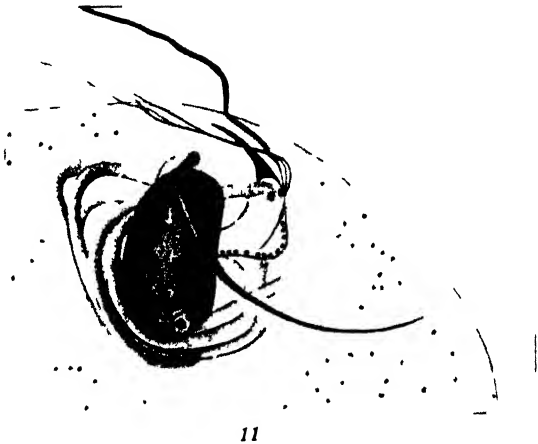
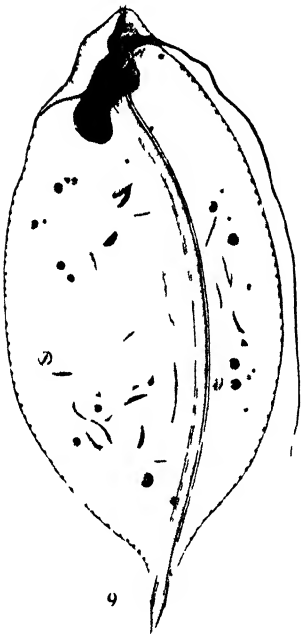
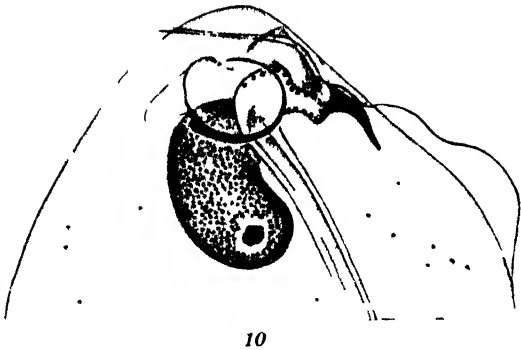


PLATE 3

Figs. 14–16. *Pseudodevescovina ramosa* new species

Fig. 14. General structure: trunk of axostyle curved within the cytoplasm (not typical; pl. 2, fig. 9, is the normal condition); parabasal apparatus, cresta, and flagella; the filament at the left of the nucleus is the end of the proximal element of the parabasal apparatus. Fl. D. $\times 1110$.

Fig. 15. Curvature of nucleus; anterior part of trunk of axostyle and capitulum; band-formed part of proximal element of parabasal apparatus. Fl. R. $\times 1830$.

Fig. 16. Parabasal apparatus: proximal element passing posteriorly to the side of the nucleus, bending forward, and passing along the level of the anterior edge of the nucleus to the periphery of the opposite side of the body; short cord arising at the bend, lying against a part of the capitulum; distal elements consisting of three cords arising close together at the bend and branching repeatedly. Fl. D. $\times 1330$.

Figs. 17–19. *Macrotrichomonas pulchra* Grassi

Fig. 17. From *Glyptotermes ceylonicus*. S. H. $\times 1830$.

Fig. 18. From *Kalotermes contracticornis*. Rodlets, probably symbionts, on the anterior part of the capitulum of the axostyle. S. H. $\times 1830$.

Fig. 19. From *Glyptotermes parvulus*, the type host. Cresta; paracrestal filament; flagella; parabasal body; blepharoplast and flagellar granules; rhizoplast; the broad clear space within the nuclear membrane is probably due to shrinkage by fixation. H. $\times 1830$.

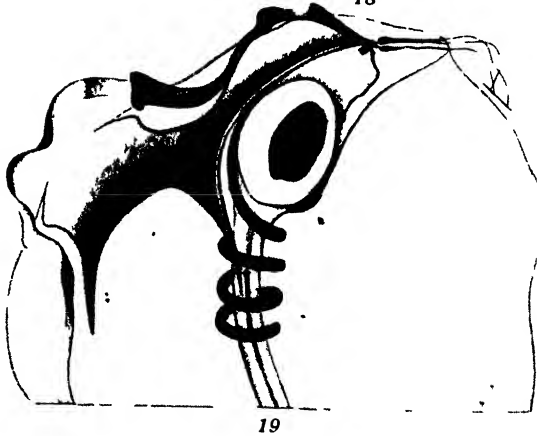
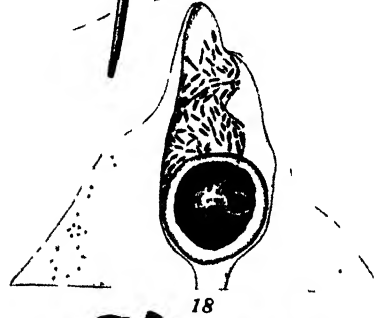
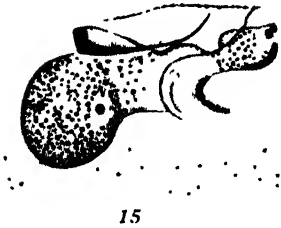


PLATE 4

Macrotrichomonas pulchra Grassi from *Kalotermes contracticornis*

Fig. 20. Flagella; anterior and posterior tufts of spirochaetes. S. H. $\times 880$.

Fig. 21. General: papilla; characteristic projection of axostyle; band-formed parabasal filament at inner edge of coiled parabasal; cresta; deeply staining margin of trailing flagellum. S. H. $\times 1830$.

Fig. 22. Parabasal body. S. D. $\times 1110$.

Figs. 23–26. Showing, from various aspects, the suspensory filament, rhizoplast, origin of anterior flagella, group of granules to which flagella and other organelles attach, paracrestal filament, cresta, parabasal body and parabasal thread, stainable inner margin of trailing flagellum. In figure 23 the suspensory lamella, to the left of the rhizoplast, is outside the curved capitulum, under both parts shown in figure. S. H. $\times 1830$.

Fig. 27. One branch originating in the anterior part of the parabasal body. S. D. $\times 880$.



PLATE 5

Macrotrichomonas pulchra from *Kalotermes contracticornis*

Fig. 28. Posterior part of parabasal body separated from short proximal part, prior to mitotic division and partly uncoiled. S. D. F. $\times 1110$.

Fig. 29. Posterior part of parabasal separated at a more posterior place; position of parts of parabasal in relation to nucleus not altered as in figure 28. This amputation probably has no relation to mitosis. S. D. F. $\times 1110$.

Fig. 30. Separation of part of old parabasal as in figure 28; new parabasal developing at one pole of the paradesmose. S. D. F. $\times 1330$.

Fig. 31. Paradesmose not shown; it is between the ends of the parabasals; the new parabasal has increased in length, and is pointed as usual during growth. S. D. $\times 1110$.

Fig. 32. Degenerating fragments of separated part of parabasal body; the pointed new parabasal has the same length as the persisting proximal part of the old one, which also probably has grown. S. D. $\times 1110$.

Fig. 33. Anaphase, with paradesmose, and chromosomes; the parabasal bodies are equal in size and alike in form. S. D. $\times 1330$.

Fig. 34. Long parabasal bodies just before plasmatomy of the flagellate into two individuals. S. D. $\times 1110$.

Fig. 35. Early prophase: flagella separated two and two at opposite poles of paradesmose; new ones not yet grown; old cresta and paracrestal filament still connected to blepharoplast. S. H. $\times 1330$.

Fig. 36. Old cresta degenerating, new ones of moderate size (only one of the two new ones is shown in the figure); the old paracrestal filament has persisted, a new one accompanies one new cresta; the old trailing flagellum, which is attached near the left end of the paradesmose, has persisted, and a new one has developed at the other end; three new anterior flagella have grown out. S. H. $\times 1330$.

Fig. 37. Early prophase: paradesmose showing two fibrils. S. H. F. $\times 1830$.

Fig. 38. Anaphase: deeper staining of paradesmose at ends; chromosomes with granules in a less heavily staining matrix. S. II. F. $\times 1830$.



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PLATE 6

Fig. 39. Early prophase: the old cresta has separated, but has not yet degenerated as it has in figure 36; two new crestas are developing (the left one in the figure is partly under the nucleus, and its anterior part, connected to the granule, is not shown in the figure); the old paracrestal filament is present on the right side; the old trailing flagellum persists on the right side, a slender new one has developed on the left side; the three anterior flagella on the right side are not shown. S. H. $\times 1830$.

Fig. 40. New crestas; old and new trailing flagella; stout paradésmose in a shallow groove in the nuclear membrane. S. H. $\times 1330$.

Fig. 41. Shortly before plasmotomy; crestas large; paradesmose greatly elongated. S. H. $\times 1330$.

Figs. 42–44. *Caduceia theobromae* França. Parabasal bodies in the prophase.
S. D. $\times 1110$.

Fig. 42. Detached part of old parabasal body degenerating, still in original position; part remaining attached pointed; no new parabasal present.

Fig. 43. Slender new parabasal developed, with same length but not same thickness as the attached part of the old one; degenerating remnants of the detached part; paradesmose in a depression of the nucleus.

Fig. 44. The two parabasals, both pointed, are of equal length, but one is still more slender than the other; the detached part of the old parabasal has broken up into granules, and much of it has been resorbed.

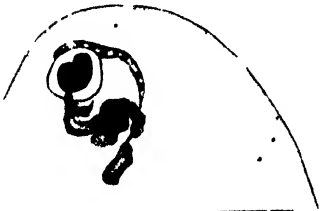
Fig. 45. *Pseudodevescovina ramosa* n. sp. Stout paradesmose; parts of newly developing axostyles; two parabasal bodies, each of which is represented by a long, deeply staining filament with some palely staining substance along it and growing out at the shoulders. S. H. $\times 1330$.



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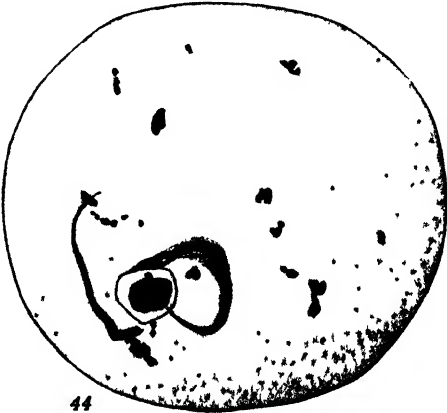
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**THE LIFE CYCLE OF
ZYGOSOMA GLOBOSUM SP. NOV.
A GREGARINE PARASITE
OF URECHIS CAUPO**

**BY
ELMER R. NOBLE**

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THE LIFE CYCLE OF ZYGOSOMA GLOBOSUM SP. NOV., A GREGARINE PARASITE OF URECHIS CAUPO

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ELMER R. NOBLE

INTRODUCTION

THE MID-GUT OF *Urechis caupo* Fisher and Maginitie is parasitized by two nonseptate gregarines. The host is an annelid worm found in the mud at low-tide mark in several bays along the coast of California. The larger gregarine inhabits the anterior part of the mid-gut, whereas the other is restricted to its posterior end. The cysts of the latter are markedly smaller than those of the former species. Consequently, there is no occasion for confusing stages in the life cycles of the two gregarines. In this paper an account is given of the morphology and complete life cycle of the first of these parasites, *Zygosoma globosum*, a new haplocyte gregarine. Particular attention will be paid to the sequence of chromosome changes throughout the cycle.

During the course of this investigation many helpful suggestions and constructive criticisms were gratefully received from Dr. Charles A. Kofoid and Dr. Harold Kirby.

MATERIALS AND METHODS

Specimens of *Urechis caupo* were taken from Drake's Bay, near San Francisco, California, and kept either in a cold room at a temperature of from 10° to 12° C. in well-aerated sea water, or at room temperature in running water. Under both of these conditions gregarine cysts commonly appeared with the fecal debris during the first three or four days. After that time the appearance of cysts was rare.

For the study of living material pieces of intestine and isolated gregarines were placed in filtered sea water and immediately examined. Cysts were usually kept in filtered, frequently changed sea water in the cold room.

Cysts were fixed for sectioning in Brasil's modification of Bouin-Duboseq fluid, Schaudinn's fluid, or Champy's fixative. The best nuclear pictures were obtained with the first of the fluids, whereas cytoplasm fixed in Champy's appeared most nearly like the living condition. Metcalf's (1908) gelatin-capsule method was used for embedding the cysts, and sections were cut at 5 or 7 microns. These were stained in Heidenhain's iron haematoxylin. Small pieces of host intestine were placed in Bouin's or Champy's fixative, sectioned at from 5 to 8 microns, and stained in Heidenhain's haematoxylin or Delafield's haematoxylin. Intestinal smears and crushed cysts were fixed in Schaudinn's fluid and stained in iron haematoxylin.

All measurements, except those of nuclei, gametes, and spores, were made from living material. The incidence of infection was one hundred per cent, and the number of individual parasites per intestine ranged from about a dozen to approximately fifteen hundred.

GENERAL MORPHOLOGY OF THE PARASITE

THE GAMONT

The mature gamont has a peculiar appearance because of the presence of nipplelike projections which cover the entire body surface except an anterior swelling in detached individuals, and a pointed posterior tip (pl. 7, fig. 1). Whether these projections have any special function is not known. The average dimensions of the adult gregarine are 300 microns in width by 400 in length. The range in width is from 200 to 380 microns; that in length from 250 to 500 microns.

The oval nucleus is always situated at approximately the center of the body. It is not visible in the living adult because of the opaque nature of the cytoplasm. The nucleoplasm is finely granular, but often the granules aggregate in small irregular groups (pl. 7, fig. 5). One large central karyosome is characteristic, but there are almost always one or more additional chromatin bodies. These usually have a stronger affinity for haematoxylin than the karyosome itself. If the karyosome is not too heavily stained, a group of vacuoles is visible within it. Often there is present within the karyosome a small, deeply staining granule (pl. 7, fig. 5). This suggests the "micronucleus" in *Diplocystis* described by Jameson (1920), but since it has not been seen in the majority of preparations its significance is questionable. The adult nucleus measures 60 by 85 microns, whereas the karyosome averages 17 microns in diameter. The long axis of the nucleus, which is in the major axis of the body, may reach a length of 120 microns.

The body of the gregarine is covered by a transparent episarc. This is a modification of the pellicle of other Protozoa, and is made up of a mass of fine supporting fibrils which run anteroposteriorly. Beneath the episarc is a layer of ectoplasm modified for support. It is about 10 microns in thickness and encloses the endoplasm, which constitutes the bulk of the body.

THE EPIMERITE

The epimerite is a large globular structure embedded in the intestinal epithelium. It averages 125 microns in diameter. Its walls are composed of episarc and ectoplasm. Instead of endoplasm there is a vacuole filled with a fluid. The fibers of the episarc tend to collect in deeply staining parallel strands with paths between, relatively free from fibers, thus giving the surface of the epimerite a ribbed appearance (pl. 7, fig. 6). In intestinal smears the whole structure may be torn out of the surrounding cells, leaving the gregarine intact.

Between the epimerite and the gamont body there is a partition which sepa-

rates the cavity of the former from the endoplasm of the latter. This partition is supported by fibrils which are continuous with the fibrils of the episarc. The method of its formation will be described below when an account of the development of the gamont is given.

CYTOLOGICAL ASPECTS

In adult individuals the cytoplasm of the anterior end stains more deeply, and appears slightly granular (pl. 7, fig. 4). This area is centrally situated, and does not extend to the periphery. It is contiguous to and just posterior to a clear area at the extreme anterior end which usually becomes devoid of cytoplasm early in development. Here we have a definite differentiation of the cytoplasm at the anterior end. This may indicate a degenerated protomerite, or perhaps an incipient one.

One other cytoplasmic differentiation of the anterior end has been noted in three individuals. In these, instead of the clear area's being devoid of any visible constituents, there are numerous deeply stainable granules which tend to arrange themselves in strands attached to the body wall at one end and hanging free at the other (pl. 7, fig. 2). One of the individuals appeared normal in all other respects. In the other two the nucleoplasm was of a homogeneous gray material and not lightly granular, as is normal. This condition of the nucleus, however, is often seen in preparations fixed in Champy's fluid. Possibly the granular strands have some phylogenetic significance along with the darkened area described above, but it is possible that they are merely artifacts.

The two gamonts in a cyst were often found to stain with a different intensity (pl. 7, fig. 7). This phenomenon led the writer to try differential staining in an attempt to bring out further cytoplasmic differences. Tests for the Golgi apparatus, chondriosomes, and paraglycogen were made.

The Golgi apparatus.—After the usual osmic acid impregnation technique was used, the two gamonts in association showed definite cytoplasmic differences. Usually one contained numerous gray bodies, whereas in the other these bodies were largely absent. In a few specimens studied, black granules were observed in one member of the pair. They were not, however, of a typical dictyosome appearance. The results with the Golgi technique are not conclusive, although evidence is given for two types of cytoplasm.

Chondriosomes.—Using the Champy-Kull technique, the writer found, in most preparations, that in one gamont in syzygy the chondriosomes were consistently more abundant than in the other. These bodies took the form of fine, deeply staining granules evenly distributed throughout the cytoplasm.

Paraglycogen.—Associated gregarines which had been fixed in 100 per cent alcohol and treated with iodine showed numerous paraglycogen bodies in their cytoplasm. These bodies were of different sizes and appeared to be more concentrated about the periphery of each gamont. One member of the pair, however, always possessed the paraglycogen in greater abundance than the other.

This evidence for sexual differences in associated gregarines in syzygy is in accord with the findings of Joyet-Lavergne (1926) and Leger and Duboscq (1903).

THE LIFE CYCLE

THE ZYGOTE AND SPOROBLAST

The account of the life cycle of this gregarine will start with the zygote because it is at this stage that each new individual begins its existence. The cycle ends with the union of gametes.

Out of approximately 150 cysts kept under various conditions in the laboratory, only one developed as far as the sporoblast stage. All the others disintegrated before gametes were fully formed. This one cyst, however, showed an abundance of zygotes. One side of the cyst contained a group of gametes and zygotes, whereas the remainder of the cyst was filled with spores (pl. 7, fig. 3). Whether or not this unequal rate of development in different parts of the cyst is the usual procedure cannot be determined, since, as has been stated, no other cysts at this stage have been observed. Nevertheless, the sequence of changes immediately preceding and following the formation of the zygote, as shown by the cyst, is, without doubt, typical for the species, regardless of whether those changes normally all take place at the same time within one cyst wall.

As soon as the gamete nuclei unite, the chromatin of each organizes itself into six rounded chromosomes averaging a little over one-half micron in diameter. Thus the first appearance of the zygote, which measures 6 by $8\frac{1}{2}$ microns, is that of a naked bit of cytoplasm containing at or near its center a nucleus with twelve distinct chromosomes. The nucleus is 3 microns in diameter. A process of synapsis immediately follows fertilization. The chromosomes are grouped to form six pairs, which are clearly discernible at this stage (pl. 8, fig. 8).

Soon after pairing, the chromosomes lose their visible individuality and merge with each other to form a mass of material in which distinct separate elements cannot be distinguished. At the same time there is an increase in the amount of chromatin, which fills the nucleus. Just before this change takes place, the zygote forms a thin resistant membrane about itself (pl. 8, figs. 9, 17). Sporoblasts average 8 by 9 microns. Their increase in size over the zygote is due to a marked vacuolization of the cytoplasm. The sporoblast nuclei are also vacuolated while in the resting condition. The zygote nucleus does not divide until after the sporocyst membrane is fully formed. The first division starts with a slight decrease in size of the nucleus. The chromatin becomes finely granular and forms itself into a knot of twelve chromosomes. The number, however, cannot be counted until division takes place. In this division there is merely a separation into two groups of six chromosomes each. The separation is not accompanied by asters or spindle formation. The nucleus elongates, becomes dumbbell-shaped, and pinches in two (pl. 8, figs. 10, 13).

This first division of the zygote nucleus is the reduction division. As will

be shown below, all divisions leading up to the formation of gametes show six chromosomes. In spite of the fact that there is a question concerning the actual count of the chromosomes in the dividing zygote, and that the writer is not certain that the last divisions before gametes are formed have been observed, it is certain that reduction is postzygotic. This certainly is based upon the fact that the zygote definitely contains twelve paired chromosomes. If reduction took place at the last division to form gametes, one would find only six and three chromosomes, while twelve would never occur. Also, six chromosomes have been observed in the divisions leading to the formation of the fully developed spore. Thus the determination of the position of the reduction division places *Zygosoma globosum* with the haploid gregarines.

The division of the zygote results in a two-nucleate sporocyst the nuclei of which, in the resting condition, are rounded and completely filled with chromatin (pl. 8, figs. 11, 14). Each of these nuclei then forms an irregular knot of six chromosomes and divides without spindle formation to form a four-celled spore (pl. 8, figs. 12, 15, 16). The cytoplasm at this stage is still highly vacuolated, and there is no indication of the separation into sporozoites. In the material studied, however, a large majority of the spores contained four nuclei. In a very few a fifth nucleus was observed, but these are probably abnormal. In the mature four-nucleate spores the nuclei migrate to the periphery and become situated at points equidistant from each other (pl. 8, fig. 16). For these reasons the normal number of sporozoites is probably four, but they have not been seen. Further evidence in support of this conclusion lies in the fact that the youngest stages found in the host epithelial cells are approximately equal in size to one-fourth of a spore.

INTRACELLULAR STAGES

Urechis caupo is probably infected by ingestion of mature spores which liberate sporozoites in the intestine. Sporozoites penetrate only epithelial cells of the anterior part of the mid-gut. The smallest intracellular stage observed was $5\frac{1}{2}$ microns in diameter. Its stained nucleus appeared as a solid black body with a tiny chromatin dot at its surface. There was no indication of a central karyosome (pl. 8, fig. 19). As the parasite grows, the parasitized cell elongates, its nucleus enlarges, and the whole structure tends to become detached from the surrounding cells except at the proximal end. The parasite swells out the distal end of the cell and the resulting tension results in a wrinkling up of the body wall of the gregarine. The nucleus at this stage takes on the characteristic appearance of the gamont nucleus. A large karyosome is surrounded by a granular nucleoplasm which stains only slightly darker than the cell cytoplasm. In addition, there are always one or more separate chromatic dots usually situated close to the karyosome (pl. 8, fig. 20).

Further growth of the parasite results in rupture of the host cell, and the parasite extends into the lumen of the gut (pl. 8, fig. 18). That portion of the gregarine which remains enclosed by epithelial tissue becomes the epi-

merite. As it grows larger, the parasitized cell becomes greatly hypertrophied. Usually epithelial cells immediately adjacent to the parasitized cell become partially separated from surrounding tissue to form a thick congested cap about the epimerite (pl. 7, fig. 4).

Pathological effects on the host are often marked. Hypertrophy of the epithelial nuclei immediately adjacent to the epimerite usually occurs. These nuclear enlargements range from a slight increase to five or six times the normal diameter (pl. 9, fig. 23). Deeply staining granules are often found both inside and around these enlarged nuclei in the cytoplasm, but the cells usually appear normal in all respects except in size (pl. 9, fig. 24).

GAMONT DEVELOPMENT

Soon after the gamont commences to grow into the lumen of the gut, the nucleus migrates posteriorly and carries with it most of the endoplasm of the

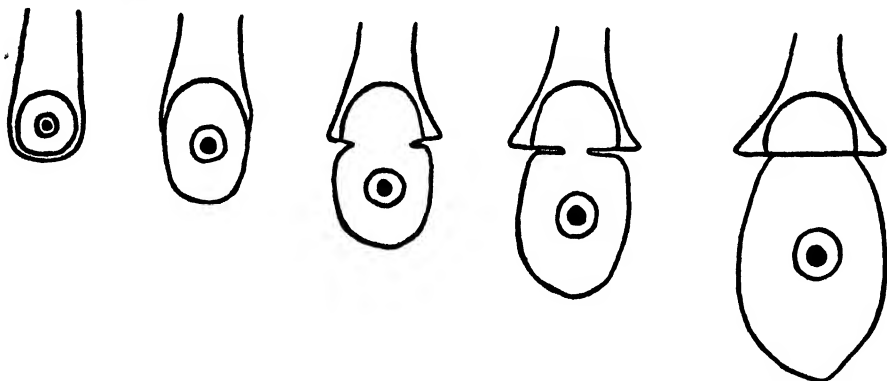


Fig. A. The formation of the epimerite and its separation from the gamont body by a partition of episarc and ectoplasm, as seen in longitudinal section.

anterior end. With further growth there occurs a gradual constriction and slight thickening of the cell wall of the gregarine at the line of junction between the body proper and the end embedded in host tissue. This process is similar to that which would take place if one were able to tie a fine thread about the gregarine along this line and slowly tighten it up. There results an ingrowing shelf which finally closes up the opening and forms a platelike partition separating the cavity of the epimerite from the larger portion of the gregarine (fig. A). This plate is made up of two layers of fibrils which radiate from a central point. The upper layer is continuous with the fibrils supporting the dome of the epimerite, whereas those of the lower layer are continuous with the fibrils of the episarc of the body wall. The latter meet at the posterior extremity of the gregarine. Both surfaces of the plate are lined with ectoplasm (pl. 9, figs. 31, 32).

SYZYGY AND CYST FORMATION

Syzygy normally occurs at an early stage of development. Very few solitary individuals possessing a fully formed epimerite separated from the gamont

body were seen. When a pair of gregarines in syzygy is attached to the host epithelium, only one member of the pair possesses an epimerite. The other is not connected to the epithelial lining. Therefore syzygy is probably accomplished by the detachment and migration of a young trophozoite to an attached individual, where it in some manner adheres to the surface. There is evidence, given by the differences which have been observed between the two gamonts of a cyst, that the migrating trophozoite is of the opposite sex from that of the stationary one.

Frequently almost all of the adult gregarines were found to be in syzygy, but there always were a number of solitary individuals. These differences in

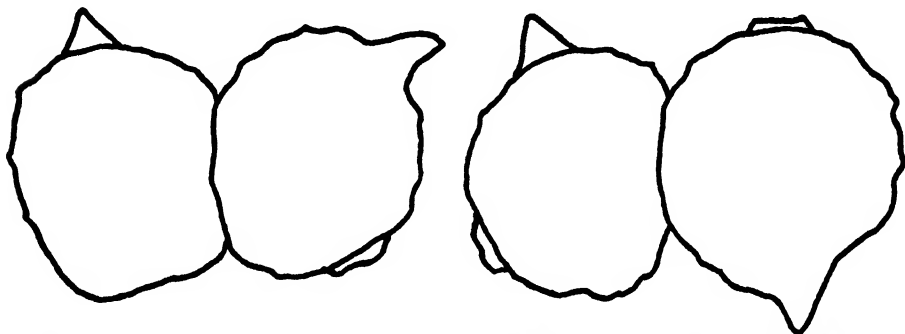


Fig. B. Two pairs of gamonts of *Zygosoma globosum* in syzygy. Both individuals of one pair are pointed in the same direction, whereas in the other pair they are in opposite directions.

the relative numbers of single and associated individuals are probably correlated with the occasional occurrence of clusters of cysts extruded at one time in the feces. Both the associated and the isolated forms are attached to the intestinal epithelium, but the largest ones readily drop off when the intestine is disturbed. Parallel syzygy is the only form of union observed. As growth continues, however, the symmetry of association is often markedly disturbed, producing pairs which appear to be attached in a haphazard fashion. The anterior ends of individuals in syzygy are pointed in the same or in opposite directions (fig. B).

If fully formed gamonts in syzygy are taken out of the intestine and placed in sea water they die without further development. They are frequently extruded with cysts in fecal material, but have never been seen to form a surrounding membrane. Thus it is evident that unless cyst formation takes place in the host intestine the detached gregarines die. For this reason the process of cyst formation has not been observed. No movement of any kind has been seen in any stage of the life cycle. Fully formed cysts may easily be detected among fecal débris without the aid of a lens. They are occasionally extruded in small clusters, but usually are solitary. Cysts are spherical to oval in shape. Two adult gregarines in syzygy secrete about themselves a thick, transparent, sticky, and gelatinous wall which averages 200 microns in thickness. The whole cyst mass averages 700 by 850 microns.

PREGAMETIC DIVISIONS

The two nuclei of a newly formed cyst are similar in appearance to the nuclei of mature gamonts. Some possess a single large karyosome, whereas in others this body has begun to break up into several pieces of different sizes. In many cases, from one to three hours after cyst formation, varying with the individual cyst and environmental conditions, fragmentation of the karyosome begins. After the nucleus is filled with numerous round, deeply staining bodies, which represent the karyosome, the nuclear wall breaks down. At this time a large spindle with two asters is formed at one edge of the nucleus, and a comparatively small fragment of the chromatin material forms itself into six chromosomes. These divide mitotically to form two groups of six chromosomes each. The earliest stage observed during this first division was a late anaphase. One end of the spindle with its chromosomes is shown in plate 9, figure 21. Only one other instance of this division was observed. In plate 9, figure 29, one may see one end of the spindle beside the disintegrating remains of the old nucleus. Plate 9, figure 22, shows the other end of the spindle of the same division. Here we see that in the daughter nucleus the chromosomes have changed to irregular groups of chromatin. The chromatin granules of the original parent nucleus become distributed throughout the cytoplasm and quickly lose their stainability. In plate 9, figure 29, one may see in the lower right-hand corner two pieces of the original karyosome which have taken less of the stain than those still at the site of the old nucleus.

The daughter nuclei of the first division are from 2 to 3 microns in diameter; these measurements are based on three nuclei only. Now there begins a period of increase in size of the nuclei, until they reach a diameter up to 12 microns. Just before the next and all succeeding divisions prior to gamete formation the nuclei may expel some of their granular contents, although usually no such phenomenon is evident. Occasionally (pl. 9, fig. 30) one finds at the side of the daughter nucleus a small accessory body. The significance of this extranuclear body has not been determined. It might arise by the development of a membrane about a few granules of chromatin which have been expelled by the "parent" nucleus. Plate 9, figure 30, represents one of the two daughter nuclei of the first division. It has attained maximum size and has begun to divide. At this stage the chromatin is still in the form of irregular clumps and strands. There is as yet no indication of chromosomes. As seen in the figure, the first indication of division is the appearance of a centrosphere situated just inside the nuclear membrane. This is differentiated as a homogeneous patch of material which stains gray with iron haematoxylin. There is no indication of a centriole. The centrosphere divides, the two products moving to opposite ends of the nucleus. The appearance of the centrosphere is accompanied by astral rays which are extranuclear. As the centro-

spheres move to opposite sides of the nucleus, the chromatin clumps up into larger bodies and collects in the center, where six chromosomes, in the form of short rods, are formed. The sequence of these changes is illustrated in plate 9, figures 25 to 28.

Leger and Duboscq (1903) stated that in *Nina gracilis* there is one extra-long chromosome from which the karyosomes of the daughter nuclei are derived. The remaining chromosomes take no part in the formation of a new karyosome. The behavior of the nuclei in the mitotic divisions of *Zygosoma globosum* suggests a similar phenomenon. Many of the anaphase figures show one pair of chromosomes which is two or three times as long as the others (pl. 10, figs. 34 and 35). However, this long chromosome cannot be traced from one division to the next, and one is unable to determine whether or not it is solely responsible for the formation of new karyosomes. In the late anaphase the chromosomes lose their identity and become broken up into granules and strands (pl. 10, fig. 36).

As the pregametic divisions proceed, the nuclei become smaller and smaller, and the chromosomes become shorter and shorter. The smallest dividing nucleus observed was 6 microns in diameter. At this stage the cyst is filled with the maximum number of nuclei, approximately twenty-five hundred (pl. 10, fig. 38).

GAMETES

Only one cyst was found at a stage between the above-described condition and fully formed gametes. This contained nuclei which averaged 3 microns in diameter. No divided forms were present, and the chromatin in the majority of the nuclei was gathered to form an irregular lobed mass in the center. Also, the cytoplasm had ceased to be a continuous matrix in which the nuclei were embedded, and had become lobed and furrowed (pl. 10, fig. 39). Each nucleus, together with a bit of surrounding cytoplasm, now separates to form an individual gamete (pl. 10, fig. 41). Gametes are round to pyriform and measure 5 microns in diameter with nuclei 2 microns in diameter. The chromatin of the nucleus of a fully formed gamete is united in one central mass. In the one cyst which showed gametes there was no indication of heterogamy. However, since the two halves of a cyst show cytoplasmic differences, as we have seen above, it is probable that differential staining of gamete material would bring out cytological differences between the two gametes which unit to form the zygote.

Gamete formation does not utilize all the cytoplasm of the cyst contents. Consequently, residual cytoplasm is present (pl. 7, fig. 3).

Fertilization occurs by the simple flowing together of two gametes (pl. 10, fig. 40). As has been stated above, the union of gametes initiates the emergence of the chromosomes, which may easily be seen in the zygote. Figure C is a schematic diagram of the life cycle of *Zygosoma globosum*.

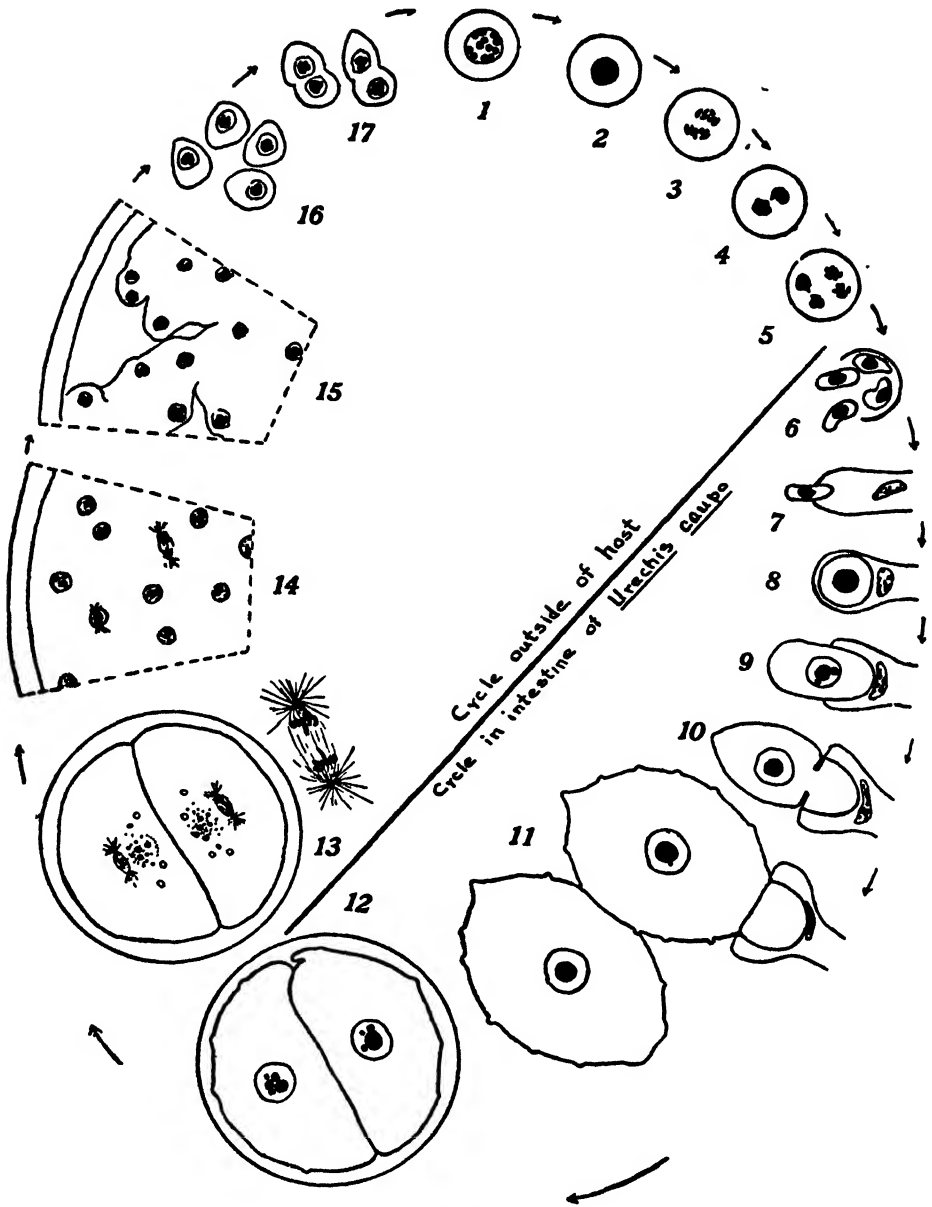


Fig. C.

For explanation see opposite page.

DIAGNOSTIC CHARACTERISTICS

Zygosoma globosum sp. nov.

A haplocyte gregarine; width 300 (200–380) microns, length 400 (250–500) microns; body of gamont covered with characteristic nipplelike processes; reduction postzygotic, haploid number of chromosomes six; epimerite a large globular knob separated from the body by a plate of supporting fibers radiating from a central point; cysts measure 360 by 400 microns without the gelatinous wall, and dehisce by simple rupture; spores roughly egg-shaped, 7 by 9 microns; four sporozoites; early development intracellular; host *Urechis caupo*; type locality Drake's Bay, California.

RELATIONSHIPS

Greeff (1880) described a gregarine from *Echiurus pallasii* taken from the coast of Norway. He observed what he supposed to be a single species, and named it *Chonorhynchus gibbosus*. This generic name was later found to be preoccupied, and Labbé (1899) renamed Greeff's parasite *Zygosoma gibbosum*. Upon examining Greeff's figures the writer was immediately impressed with the striking similarity between the supposed young stages and the second species of gregarine found in *Urechis caupo*. They are also almost identical in appearance with one of two species of gregarines parasitic in *Urechis unicinctus*. This similarity, and the fact that Greeff did not work out the life cycle, or even mention the epimerite or cysts, is strong evidence that he was dealing with two distinct species. The larger one of these, his "adult," clearly belongs, as we shall see below, in the same genus as the parasite described in this paper. Consequently, the generic name *Zygosoma* has been retained for the larger of the two gregarines of *Urechis caupo* because Greeff, in his description, mentioned and figured the adult stage first.

The gamonts of both *Zygosoma gibbosum* and *Z. globosum* are covered with nipplelike projections which are characteristic of, and peculiar to, the genus. Greeff states that in *Z. gibbosum* the adult organisms are almost always asso-

Fig. C. A schematic diagram of the life cycle of *Zygosoma globosum*. Stages represented are not drawn in proportion to their normal size.

1. Diploid zygote after fertilization, showing twelve chromosomes grouped in six pairs.
2. Zygote after formation of sporoblast membrane.
3. The reduction division, showing the separation of the twelve chromosomes into two haploid groups of six each.
4. The product of the reduction division. A two-celled spore.
5. A spore with four nuclei.
6. Liberation of four sporozoites within the intestine of the host.
7. Sporozoite entering epithelial cell of host.
8. Growth of parasite within epithelial cell of host into trophozoite.
9. Rupture of epithelial cell, and elongation of parasite into lumen of intestine.
10. Development of epimerite.
11. Syzygy of adult gamonts.
12. Fully formed fertilization cyst.
13. First division of gamont nuclei in cyst, with enlarged figure of one of the mitotic spindles showing six chromosomes.
14. Pregametic nuclei.
15. Lobed cytoplasm before formation of gametes.
16. Gametes.
17. Fertilization: fusing haploid gametes.

ciated, being attached end to end, and are filled with small clear vacuoles. They have a length and breadth of one millimeter, and show the typical slow, gliding gregarine movement. The nucleus is excentric in position. On the other hand, the gamonts of *Z. globosum* are often solitary, are attached side by side in syzygy, and have no such vacuoles. They measure only 300 by 400 microns, do not glide about, and their nucleus is central in position. Another point of difference is that the gregarines from *Echiurus* are especially abundant in the spring, whereas those of *Urechis* are more abundant in the fall.

The only other closely related gregarine is that described by Iitsuka (1933) from *Urechis unicinctus*. This parasite is 90 microns in diameter, and has "wavy or warty projections" on the body surface which are comparable to the nipplelike projections mentioned above. The gamonts are attached side by side in syzygy. The epimerite of Iitsuka's gregarine is similar in shape to that of *Zygosoma globosum*, and gametes and spores of the two are approximately the same in appearance and size. Mature spores of the Japanese form, however, contain eight sporozoites, whereas those of *Z. globosum* contain only four. Iitsuka did not mention the work of Greeff, and placed his gregarine in the genus *Lecudina*, giving it the specific name of *fluctus*.

All the species of *Lecudina*, except *L. fluctus*, are solitary gregarines without a warty surface. There is no doubt that *Lecudina fluctus*, *Zygosoma gibbosum*, and *Z. globosum* all belong to the same genus. I propose, therefore, to remove Iitsuka's gregarine from the genus *Lecudina* and place it in the genus *Zygosoma*, which is clearly distinct from all other genera of the family Lecudinidae Kamm.

Another described gregarine may possibly be related to *Zygosoma globosum*. Mackinnon and Ray (1931) described a gregarine, *Hyperidion thalassemae*, from the intestine of *Thalassema neptuni* Gartner, a worm closely related to *Urechis*. The most striking point of resemblance is in the method of attachment to the host tissue. Indeed, their figure 3 (pl. 22) could easily be taken for one of *Zygosoma globosum* if there were a partition between the portion of the parasite surrounded by the epithelial cell and the remainder of the body. In describing the parasitized cell the authors say that "not only does it become hypertrophied, but it rises up beyond its fellows until it swings clear of the general level of the epithelium and hangs into the gut, though it remains tethered to the wall by four or five rooting protoplasmic threads." This is very similar to what takes place in the gut of *Urechis caupo*.

Since Mackinnon and Ray did not describe the life cycle, they could not ascertain the systematic position of their gregarine. They did mention, however, that Greeff's gregarine was the only one that they could find that bore even the slightest resemblance to theirs.

SYSTEMATIC POSITION

The genus *Zygosoma* belongs in the family Lecudinidae Kamm, which is the first of four families listed by Bhatia (1930) in the tribe Heteropolaridea. This family is characterized by trophozoites with an epimerite which is symmetrical, simple, and deformable. The sporocysts are oval, with a thickening at one pole.

Following is a list of the described species of the genus *Zygosoma* with their synonyms.

Genus *Zygosoma* Labbé, 1899

Body nonseptate and covered with nipplelike or wavy projections; epimerite a simple knob; spores oval. Intestine of marine annelids.

Zygosoma gibbosum (Greeff) Labbé

1880. *Chonorhynchus gibbosus* Greeff

1899. *Zygosoma gibbosum* (Greeff) Labbé

Sporonts always associated; surface of body covered with conical and raised processes; sporonts filled with clear vacuoles; nucleus excentric; length and breadth one millimeter; cysts and spores unknown.

Zygosoma fluctus (Iitsuka) Noble

1933. *Lecudina fluctus* Iitsuka

1938. *Zygosoma fluctus* (Iitsuka) Noble

Sporonts associated; body covered with wavy projections; size 400–700 by 90 microns; epimerite a knoblike protuberance at the anterior end; cysts dehiscence by simple rupture; spores oval, 8 by 9 microns; eight sporozoites.

Zygosoma globosum Noble

With characters as given under "Diagnostic Characteristics."

DISCUSSION

CYTOLOGY

The most important contributions to the study of cytoplasmic sexualization have been made by Joyet-Lavergne (1926). His main conclusions are as follows: The Golgi apparatus and chondriosomes are more abundant in the male than in the female gamont, whereas reserve albuminoids and paraglycogen are more abundant and form larger granules in the female individuals. Also, in the male the hyaloplasm is more compact than in the female.

Naville (1931) is of the opinion that so-called isogamous forms are actually masked anisogamous forms, and that if a careful comparison is made between the cytological aspects of two gamonts in syzygy, or the two halves of a young cyst, this anisogamy becomes apparent.

The differences between paired gamonts of *Zygosoma globosum* lend support to Naville's hypothesis. As we have seen, the two gamonts of a cyst stain with different intensities with iron haematoxylin. Also, tests for Golgi bodies, chondriosomes, and paraglycogen show the same distribution, in general, as that described by Joyet-Lavergne. These differences give evidence for heterogamy in *Zygosoma globosum* in spite of the fact that no differences between two gametes in the process of fertilization have been observed.

SYNAPSIS

Naville (1927) described a pairing of chromosomes in the zygote of *Urospora lagidis*. This is noteworthy because reduction in *Urospora lagidis* is gametic. Thus there is a time interval between synapsis and reduction. Naville believes that this is an intermediate condition between those forms in which reduction is postzygotic and those which have gametic meiosis. Naville (1930) also observed what he supposed to be a synapsislike arrangement of chromosomes in the zygotes of three species of *Monocystis*. In verification of this view, Naville points out that the figures of Calkins and Bowling (1926) of the zygote of a species of *Monocystis* show very definitely a pairing of chromosomes. These species of *Monocystis* mentioned above all have gametic meiosis.

So far as the writer knows there has been no mention of chromosome pairing in any of the described gregarines with postzygotic meiosis. Jameson (1920) has made the most careful study of chromosome changes in a gregarine of this category, but he did not observe a pairing of chromosomes in the zygote. Also, his figures of this stage give no suggestion of synapsis. Thus the work on *Zygosoma globosum* is the first reported instance of a definite synapsis in a nonseptate gregarine with zygotic reduction.

SUMMARY

An account has been given of the life cycle of *Zygosoma globosum* sp. nov., a nonseptate gregarine parasitic in the intestine of *Urechis caupo* Fisher and Maginitie. Particular attention was paid to the chromosome cycle of the parasite. The body of the gamont is covered with nipplelike projections. A large globelike epimerite is present which is separated from the body by a partition of fibrils radiating from the center. Syzygy occurs at an early stage of development. Points of particular importance in the life cycle are the following: Twelve chromosomes become visible immediately after the union of gametes. The first division of the zygote is the reduction division, two groups of six chromosomes each separating out without the appearance of asters or a spindle. The following divisions to form four sporozoites are different from the zygotic division in that only six equational chromosomes are involved. Sporozoites penetrate epithelial cells of the host. Intracellular development is short-lived, the trophozoite breaking out of the distal end of the cell. That portion of the gregarine which remains enclosed by epithelial tissue becomes the epimerite. Cysts are extruded with the feces of the host. The first mitotic division starts with a fragmentation of the karyosome. Only a small portion of the nucleus is concerned with the formation of the first daughter nuclei. In all the pregametic divisions there are six chromosomes. These divisions are accompanied by long astral rays and spindle fibers. Gametes appear isogamous, but differential staining would probably bring out cytoplasmic differences.

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida. The following abbreviations are used: B., Bouin's fluid; BB., Brasil's modification of Bouin-Duboscq fluid; C., Champy's fluid; S., Schaudinn's fixative. All preparations were stained in Heidenhain's iron haematoxylin, unless otherwise noted.

PLATE 7

Fig. 1. Adult gamont of *Zygosoma globosum*. Drawn from life. $\times 92$.

Fig. 2. Beaded strands of deeply stained material in the clear area of the anterior end of a gamont. C. $\times 350$.

Fig. 3. Portion of cyst showing distribution of gametes, zygotes, and spores. The darker bodies are the gametes and newly formed zygotes. There is also at the left some residual cytoplasm. S. $\times 111$.

Fig. 4. Longitudinal section of adult gamont attached to host epithelium. S. $\times 92$.

Fig. 5. Nucleus of adult gamont showing pale karyosome and three other darker chromatic granules. S. $\times 1666$.

Fig. 6. Portion of epimerite edge showing fibrils of episarc grouped into heavily staining strands. S. $\times 180$.

Fig. 7. Section of young cyst before nuclear division. Note difference of staining reaction of the two sides. S. $\times 40$.

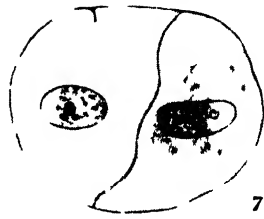
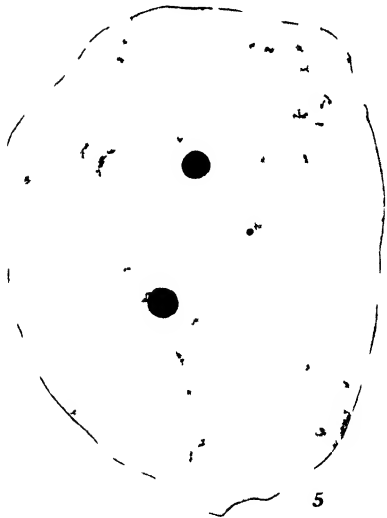
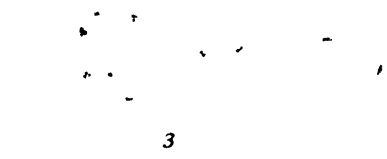


PLATE 8

Fig. 8. Zygotes immediately after fertilization. Three of them show twelve paired chromosomes. S. \times 1888.

Fig. 9. Formation of sporocyst membrane about zygote. S. \times 1888.

Fig. 10. Beginning of the reduction division. S. \times 1888.

Fig. 11. Daughter nuclei from reduction division. S. \times 1888.

Fig. 12. The last division to form a four-nucleate spore. One pair of daughter nuclei showing six chromosomes. S. \times 1888.

Fig. 13. Reduction division. S. \times 1888.

Fig. 14. Typical appearance of dividing spore nuclei. S. \times 1888.

Fig. 15. Typical appearance of dividing spore nuclei. S. \times 1888.

Fig. 16. Mature spore. S. \times 1888.

Fig. 17. Three zygotes with sporocyst membrane. S. \times 1888.

Fig. 18. Young trophozoite breaking out of distal end of host epithelial cell. S. \times 380.

Fig. 19. Young intracellular stage. S. \times 1888.

Fig. 20. Older intracellular stage. Note difference of nucleus from that in figure 19. S. \times 1888.



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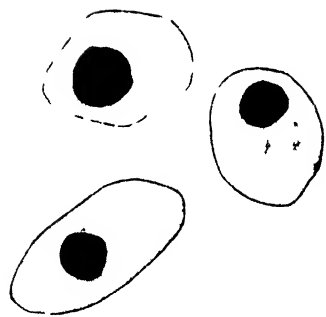
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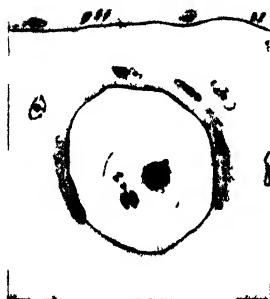
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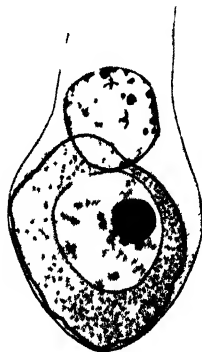
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PLATE 9

Fig. 21. Anaphase of the first division of the gamont nucleus in the cyst. Note six chromosomes. S. $\times 1888$.

Fig. 22. Telophase of the first division. BB. $\times 1888$.

Fig. 23. Hypertrophied epithelial nuclei about an epimerite. S. $\times 92$.

Fig. 24. Granules in and about the hypertrophied epithelial nuclei. S. $\times 92$.

Figs. 25-28. Sequence of mitotic changes in pregametic divisions. BB. $\times 1888$.

Fig. 29. The other end of the telophase figure shown in figure 22. Note the disintegrating remains of the gamont nucleus. BB. $\times 944$.

Fig. 30. One of the daughter nuclei of the first division in the cyst. Note the extranuclear body and the intranuclear centrospheres. BB. $\times 1888$.

Fig. 31. The partition between the epimerite and the gamont body. Note the fibrils radiating from a central point. S. $\times 575$.

Fig. 32. A cross section of the epimerite just anterior to the partition. Note episarc threads grouped in strands. The whole structure is surrounded by the epithelial cell. B. $\times 185$.



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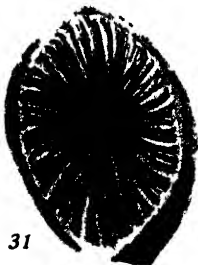
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PLATE 10

Fig. 33. Beginning of anaphase of a pregametic division. BB. $\times 1888$.

Figs. 34–35. Late anaphase of pregametic divisions. Note that one of the six chromosomes is much longer than the others. BB. $\times 1888$.

Fig. 36. Beginning of telophase of pregametic division. BB. $\times 1888$.

Fig. 37. End of telophase of pregametic division.

Fig. 38. Portion of cyst filled with pregametic nuclei. BB. $\times 944$.

Fig. 39. Lobed cytoplasm just prior to gamete formation. C. $\times 1888$.

Fig. 40. Fertilization. BB. $\times 1888$.

Fig. 41. Gametes. Two have not yet been budded from the cytoplasm. BB. $\times 1888$.



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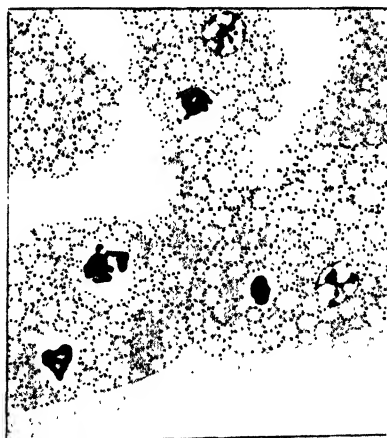
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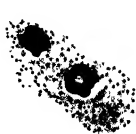


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**NEW SUBGENERA AND SPECIES
OF DIAPTOMID COPEPODS
FROM THE INLAND WATERS
OF CALIFORNIA AND NEVADA**

**BY
S. F. LIGHT**

**UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Volume 43, No. 3, pp. 67-78, 23 figures in text**

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NEW SUBGENERA AND SPECIES OF DIAPTOMID COPEPODS FROM THE INLAND WATERS OF CALIFORNIA AND NEVADA

BY
S. F. LIGHT

FOUR OF THE SIX new species described herein* are from California, two from northern Nevada. For the two Nevada species and for other collections from that area I wish to express my indebtedness to my colleagues Professor Alden H. Miller and Dr. Richard M. Eakin. Other acknowledgments will be found in the text. Syntypes have been deposited in the United States National Museum, Washington, D. C., as noted in the text; other types are in my collection.

I am allocating the six species to *Diaptomus* Westwood, but erecting three new subgenera to include them, postponing to a later paper (a revision of the American species) a statement of my reasons for believing that these subdivisions of *Diaptomus* are of subgeneric rather than generic rank.

Subgenus *Hesperodiaptomus* new subgenus

Type species.—*Diaptomus eiseni* Lilljeborg, 1889.

Diagnosis.—Distal process of terminal segment of exopodite of left fifth leg of male short, digitiform; lateral process a flattened, curving sharp-pointed spine with finely setose edges (fig. 23). Endopodite of fifth leg of female with two long, straight, equal or sub-equal apical setae (fig. 4). Spine on outer margin of exopodite of right fifth leg of male near distal end of second segment (fig. 5); spine straight or somewhat sinuous, finely serrated on inner margin of distal half (figs. 5, 7). Major articulated spines present only on segments 10, 11, and 13 of right antennule of male (fig. 2). Inner border of apical segment of exopodite of left fifth leg of male swollen to form two pads (fig. 23), the distal pad set with short, heavy spinelets, the posterior with slender hairs. Third segment of exopodite of fifth leg of female distinct, bearing a seta and a spine, the second segment also bearing a spine. Process on antepenultimate segment of right antennule of male usually longer than penultimate segment, tapering. Species relatively large with short urosomes.

The five species which I consider to belong to the new subgenus are listed below:

- D. caducus* new species
- D. eiseni* Lilljeborg, 1889
- D. franciscanus* Lilljeborg, 1889
(including *Diaptomus bakeri* Marsh, 1907)
- D. nevadensis* new species
- D. shoshone* Forbes, 1893

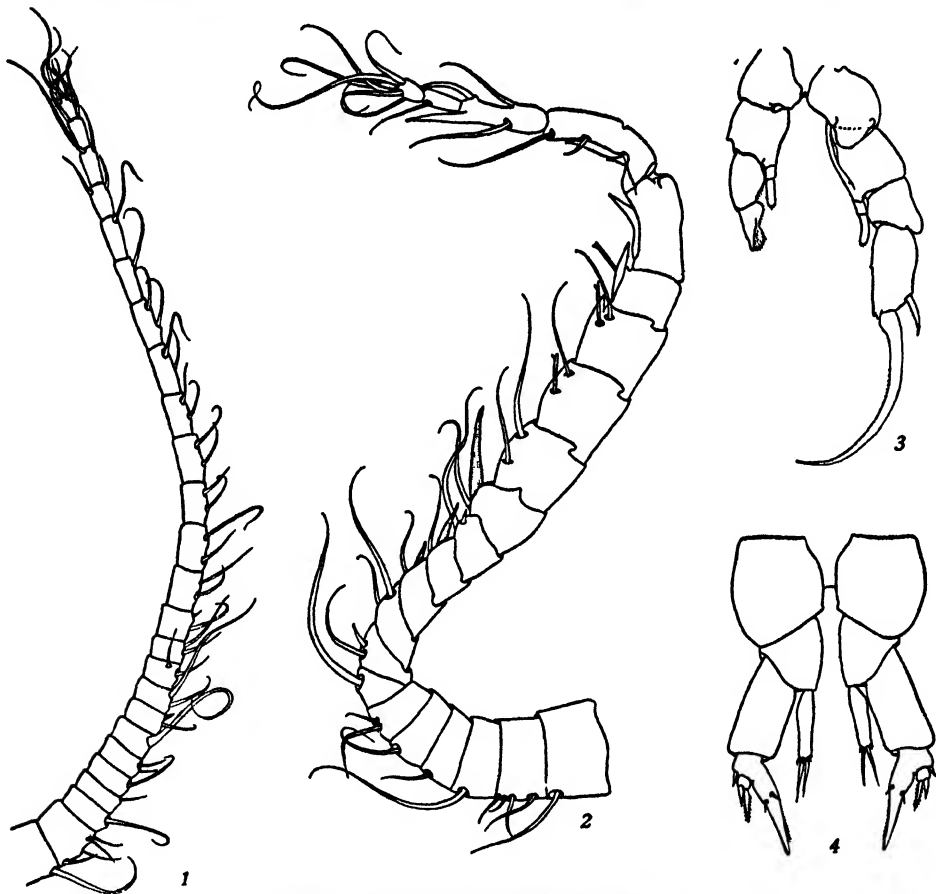
Diaptomus caducus new species (figs. 1-5, 23)

Large, females about 2.8-3.3 mm. long, exclusive of furcal setae, males about 2.45-2.65 mm. Metasome plump, strongly convex in dorsal profile, especially at anterior and posterior ends; last segment of metasome partially separate; posterolateral projections large, reaching beyond spines of genital segment; projections directed obliquely posteriorly and laterally,

* This study has been carried out with the assistance of the Works Progress Administration.

each tapering to sharp hyaline points tipped by tiny spines; a second smaller point and spine on dorsal margin.

Antennules (fig. 1) stout, relatively short, reaching to end of metasome. Small setae unmodified (figs. 1, 2); setae unusually numerous (figs. 1, 2), four setae on segment 2, two each on segments 6, 9-11, 13-19, 22-24, one each on all others save segment 25, which has five. Major spines on segments 10, 11, and 13 of right antennule of male (fig. 2); major spines straight to somewhat incurved; spine of segment 10 less than half as long as width



Figs. 1-4. *Diaptomus caducus* new species.

Fig. 1. Right antennule of female. ($\times 52$.)

Fig. 2. Right antennule of male. ($\times 69$.)

Fig. 3. Fifth legs of male in posterior view.

($\times 69$.)

Fig. 4. Fifth legs of female. ($\times 85$.)

of antennule, that of segment 11 somewhat more than half as long as width of antennule, and that on segment 13 much longer, at least twice as long as that on segment 11. Segments 14-16 of antennule markedly swollen, segments 13 and 17 somewhat less so; segment 16 with short, distally directed spinous process near distal end; process on antepenultimate segment (fig. 2) longer than penultimate segment, variable in details of shape, but always thick at base and usually tapering to point, rarely with swollen tip.

Fifth legs of male (figs. 3, 5) narrow, somewhat elongated; left leg not reaching to middle of second segment of exopodite of right. First basal segment of right leg with a variably shaped, distally directed lamella arising from lateral half of distal margin of posterior face of segment (fig. 3). First segment of right exopodite with thick projection on distal lateral corner and hyaline projection on distal medial corner (fig. 5). Second segment

of right exopodite parallel-sided, about twice as long as wide, with minute, low spine on posterior face near center of lateral margin (fig. 3); lateral spine, nearly as long as segment is wide (fig. 3), arising from distal lateral angle, continuing almost in line with lateral margin of segment, or forming a slight angle with it; lateral spine sharply narrowed from middle; distal half often somewhat incurved, slender, tapering to fine, sharp tip; claw (figs. 3, 5) nearly as long as rest of exopodite plus second basal segment, relatively slender, with fine serrate median margin. Second basal segment of left leg twice as broad as first segment of exopodite; lateral margin distinctly notched distally at point of origin of lateral hair. Basal segment of left exopodite (fig. 23) swollen, with constricted distal end; distal segment shorter than basal segment; basal pad of distal segment large, projecting medially, its hairs relatively short; distal pad smaller, anteriorly directed, its spinelets relatively slender, somewhat recurved, distally directed. Processes of distal segment short; terminal process blunt to pointed, usually shorter than distal extension of distal pad; proximal process (fig. 23) a short, flat spine with very broad base, hardly curved or inbent, extending but slightly beyond pad in lateral view. Endopodites (figs. 3, 5) short, extending but little beyond first segment of exopodites, usually two-segmented; right endopodite slender throughout; left slender and two-segmented (fig. 3) or larger, swollen near middle or grooved and unsegmented (fig. 5); each bearing at distal end a group of short, needlelike hairs.

Fifth legs of female (fig. 4) relatively long and slender; third segment of exopodites small but distinct, its spine a little more than half as long as its seta; spine on second segment somewhat more than half as long as spine on third segment. Second segment with claw nearly as long as outer margin of first segment; claw relatively slender, somewhat curved, its anterior surface marked by a marginal line of spinelets (teeth) running along the medial margin from the distal third to the basal third; lateral margin with a single small spine (fig. 4) or a short line of spinelets. Endopodite more or less distinctly two-segmented, reaching nearly to end of first segment of exopodite, with convex lateral and straight inner margins; apical portion of endopodites truncated, bearing two long apical setae, the median about four-fifths as long as the lateral, and with a group of hairs graduating into distal spinelets.

Syntypes.—U. S. Nat. Museum, no. 72566.

Taxonomic position.—The large number of setae on the antennules distinguishes this species from any other diaptomid known to me, and the absence of the slender apical extension of the endopods of the fifth leg of the female, together with numerous minor characters, distinguishes it from otherwise closely related species, such as *D. eiseni* Lilljeborg and *D. shoshone* Forbes.

Distribution.—*H. caducus* has been taken in five localities, all in central California. Three of these are in or near the San Francisco Bay region, namely, two permanent ponds in Oakland, Alameda County, seasonal ponds in Richmond, Contra Costa County, and Lake Lagunitas on the campus of Stanford University, San Mateo County (A. B. Burch and R. E. Smith). One collection was made from a pond near Sacramento in the central valley of California (E. A. Andrews), and one from a seasonal pond near Granite Lake in the Sierra, Amador County (J. Ashley), at an elevation of 6800 feet. All collections were made in early spring, save that at Granite Lake, which was taken on June 23.

Diaptomus nevadensis new species
(figs. 6 and 7)

Female unknown. Male about 3.5 mm. long, exclusive of the furcal setae. Fifth somite of urosome and furcal rami asymmetrical, the former shorter than the latter.

Left antennule of male with usual setal arrangement, three on segment 2, two each on 9, 11, and 22–24, one each on other segments; small setae somewhat modified, sinuous. Major

spines on segments 10, 11, 13 of right antennule; major spines slender, that on segment 11 longest, other two subequal, somewhat more than half as long. Segment 15 of right antennule with minute spinous process in front of middle; segment 16 with larger, more distally placed process; spinous process on antepenultimate (23d) segment considerably longer than penultimate segment (fig. 6), distally slender, obliquely directed, outcurved beyond middle.

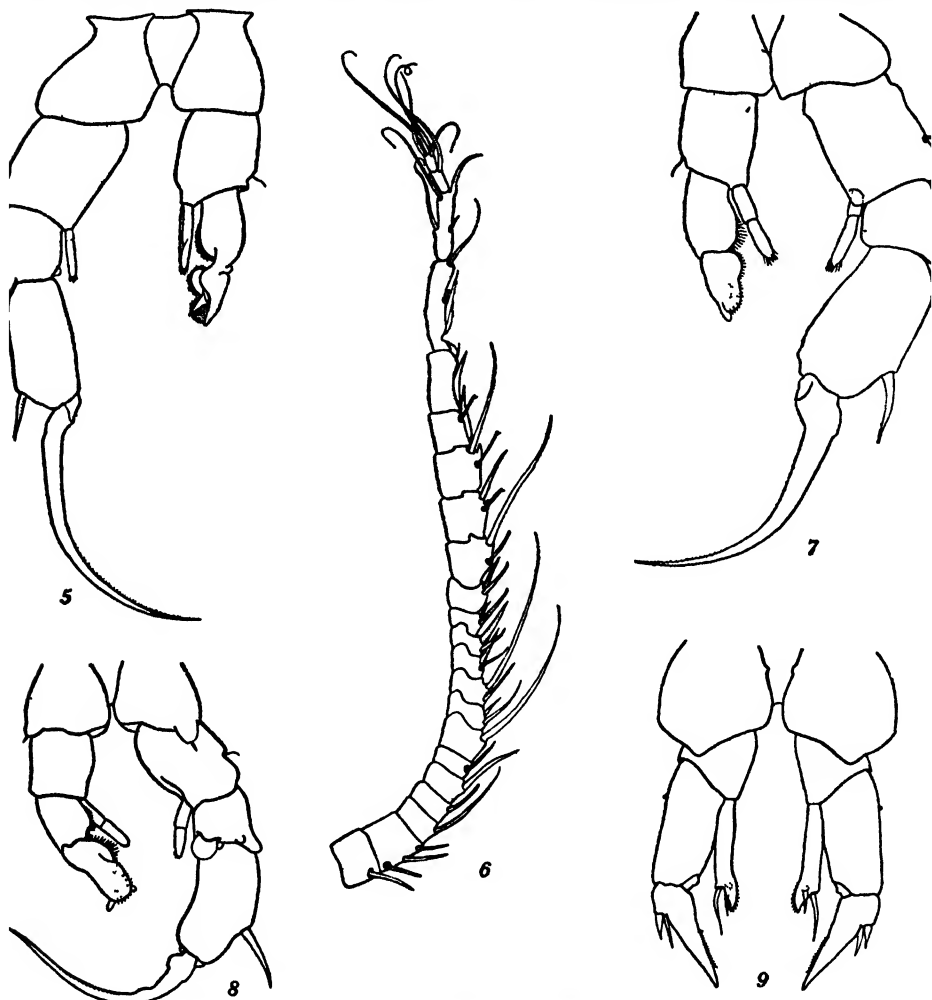


Fig. 5. Fifth legs of male of *Diaptomus caducus* new species in anterior view. ($\times 98$.)

Fig. 6. Right antennule of male of *Diaptomus nevadensis* new species. ($\times 83$.)

Fig. 7. Fifth legs of male of *D. nevadensis* in posterior view. ($\times 83$.)

Fig. 8. Fifth legs of male of *Diaptomus natriophilus* new species in posterior view. ($\times 165$.)

Fig. 9. Fifth legs of female of *D. natriophilus*. ($\times 165$.)

Fifth legs of male (fig. 7) somewhat elongated; left leg reaching about to middle of second segment of right exopodite; claw somewhat longer than rest of exopodite. Basal segment of right leg short and broad, with oblique, convex lateral margin; second basal segment straight-sided, longer than wide; inner margin of first segment of right exopodite about half as long as width of segment, outer margin about as long as segment is wide; distal lateral corner extended into a bluntly pointed process; second segment about twice as long as broad, inner margin approximately straight, outer constricted at either end; spine slightly in front of distal third, not quite as long as width of segment, somewhat

curved, directed laterally and distally, distinctly serrated on distal face. Distal segment of exopodite of left fifth leg of male (fig. 7) shorter than first segment; basal pad of inner surface distinct but not especially prominent; outer margin of segment somewhat convex, notched at base of distal process; processes short; distal process digitiform, thick, projecting distally; proximal process a curved spine, not projecting beyond margin of distal pad. Endopodites reaching beyond basal segment of exopodite; each ending in a very sharp, spinelike tip; left exopodite distinctly two-segmented, right less distinctly so.

Syntypes.—U. S. Nat. Museum, no. 72570.

Taxonomic position.—The subterminal position of the spine on the second segment of the exopodite of the right fifth leg of the male distinguishes this species from the other species of the subgenus.

Distribution.—Taken only once, in a broad, shallow, muddy lake, probably alkaline and seasonal, in Nevada, about 10 miles south of Denio, Oregon, elevation about 6000 feet, on June 8, 1935, by Professor A. H. Miller and Dr. Richard M. Eakin. *Diaptomus spinicornis*, another new species, described below, was collected with it.

Subgenus *Leptodiaptomus* new subgenus

Type species.—*Diaptomus siciloides* Lilljeborg, 1889.

Diagnosis.—Distal process of last segment of exopodite of left fifth leg of male terminal, distally directed; proximal process medially directed; both processes short and digitiform (figs. 21, 22); lateral process often with a few minute blunt setae on proximal face. Endopodite of fifth leg of female with two short, usually basally curved, stiff, spinelike setae (figs. 9, 13). Spine on outer margin of exopodite of fifth leg of male relatively short and weak and curved (fig. 17). Major spines always present on segments 10, 11, and 13 of right male antennule (fig. 14) and rarely on 8 (*D. tyrrelli* and *D. connexus*, fig. 19). Inner surface of apical segment of exopodite of left fifth leg of male armed as in *Hesperodiaptomus* but usually without deep constriction between two areas. Third segment of exopodite of fifth leg of female obsolete, its seta and spine persistent (fig. 18), grouped with the spine of the second segment when present. Antepenultimate segment of right antennule of male with hyaline lamella or more commonly a spinous process varying in nature and size (figs. 10, 11, 19) but usually shorter than penultimate segment. Species small to medium sized.

The subgenus includes numerous species which fall into several natural groups. The species are listed alphabetically below.

- Diaptomus ashlandi* Marsh, 1893
- D. connexus* new species
- D. minutus* Lilljeborg, 1889
- D. mexicanus* Marsh, 1929
- D. novamexicanus* Herrick, 1895
- D. natriophilus* new species
- D. nudus* Lilljeborg, 1889
- D. sicilis* Forbes, 1882
- D. siciloides* Lilljeborg, 1889
- D. signicauda* Lilljeborg, 1889
- D. spinicornis* new species
- D. tenuicaudatus* Marsh, 1905
- D. tyrrelli* Poppe, 1888
- D. washingtonensis* Marsh, 1907

Diaptomus natriophilus new species

(figs. 8-10)

Male about 1.50 mm. long, female about 1.9 mm., exclusive of furcal setae; urosome relatively long and slender; urosome of male tapering, last segment not much more than half as wide as first; segments subequal in length.

Antennules of female and left antennule of male with usual setal formula, three on second segment, two each on 9, 11, 22-24, one each on all others except segment 25, which has five; modified setae stiff, weakly sinuous, slender, especially in distal third, present on segments 8, 9, 12, 13, 15, 17, 19, 20, and 22. Right antennule of male (fig. 10) not conspicuously swollen; major spines on segments 10, 11, and 13; spines relatively slender, somewhat out-curved, the one on segment 11 longest and stoutest, that on segment 13 about as long as the one on segment 10; no spinous processes on swollen portion; process on segment 23 (fig. 10) longer than penultimate segment, relatively slender, of about the same thickness from base to near tip, where it is grooved and spatulate.

Fifth legs of male (fig. 8) as in the *siciloides* group. First segment of right exopodite longer on outer face than wide, considerably shorter on inner face, extended at distal lateral corner into a blunt, distally directed lobe flanked medially on the posterior face by a shorter, flatly rounded lamella and bearing on its posterior face near the medial distal corner a blunt, tongue-shaped, hyaline lamella projecting somewhat distally and medially and overlying a small, thick, transversely elongated knob. Second segment of exopodite hardly twice as long as wide, with angularly convex outer margin and concave inner margin; spine weakly curved, about as long as width of segment, situated just in front of middle, serrate in distal half of distal face; claw somewhat longer than rest of exopodite, strongly curved, tip recurved. Right endopodite short, reaching to, or slightly beyond, proximal margin of second segment of exopodite; left endopodite clearly two-segmented, reaching to middle of second segment of exopodite. Two segments of left exopodite (fig. 8) subequal; processes of distal segment short, subequal, digitiform, both somewhat obliquely directed. Fifth legs of female as in figure 9; first segment of exopodite nearly half as wide as long, lateral margins faintly convex with minute tubercle at basal third; inner margin of first segment of exopodite about equal to second segment with its claw, slightly shorter than endopodite. Third segment obsolete, its seta twice as long as its spine; spine of second segment obsolete; claw nearly straight, bearing teeth, as indicated in figure 9; endopodite slender, with relatively long apical portion; apical setae distinctly unequal, stiff, curved, projecting obliquely.

Syntypes.—U. S. Nat. Museum, no. 72569.

Taxonomic position.—Although *D. natriophilus* belongs to the *siciloides* group, it is a distinct species, large for the group, and is easily recognizable because of the spatulate process on the antepenultimate segment of the right antennule of the male.

Distribution.—*D. natriophilus* was originally collected by me in early June from Lake Baldwin at an elevation of 6670 feet in the San Bernardino Mountains of southern San Bernardino County, California. The water was saturated with salts, reportedly, in part at least, sodium salts. Like other species taken in alkaline situations, it was bright red in color. It has since been taken in five other localities in California: a dwindling irrigation seepage pond in Stanislaus County in the San Joaquin Valley; Little Borax Lake, Lake County, at an elevation of about 1200 feet; Topaz Lake (Alkali Lake on U. S. Geological Survey Map) partly in Alpine County and partly in Nevada at an elevation of 4900 feet; a reservoir near Big Pine, Inyo County, elevation about 3800 feet, and Lake Diaz near Lone Tree, Inyo County, elevation 3700 feet. The Topaz Lake collection was one of a large number made in connection with

the trout food survey and kindly put at my disposal by Dr. P. R. Needham of the United States Bureau of Fisheries. The two collections from Inyo County were among a number kindly made for me by Dr. A. E. Michelbacher, of the University of California Experiment Station. Lake Baldwin, Little Borax Lake, and Lake Diaz were strongly alkaline, the others probably more or less so.

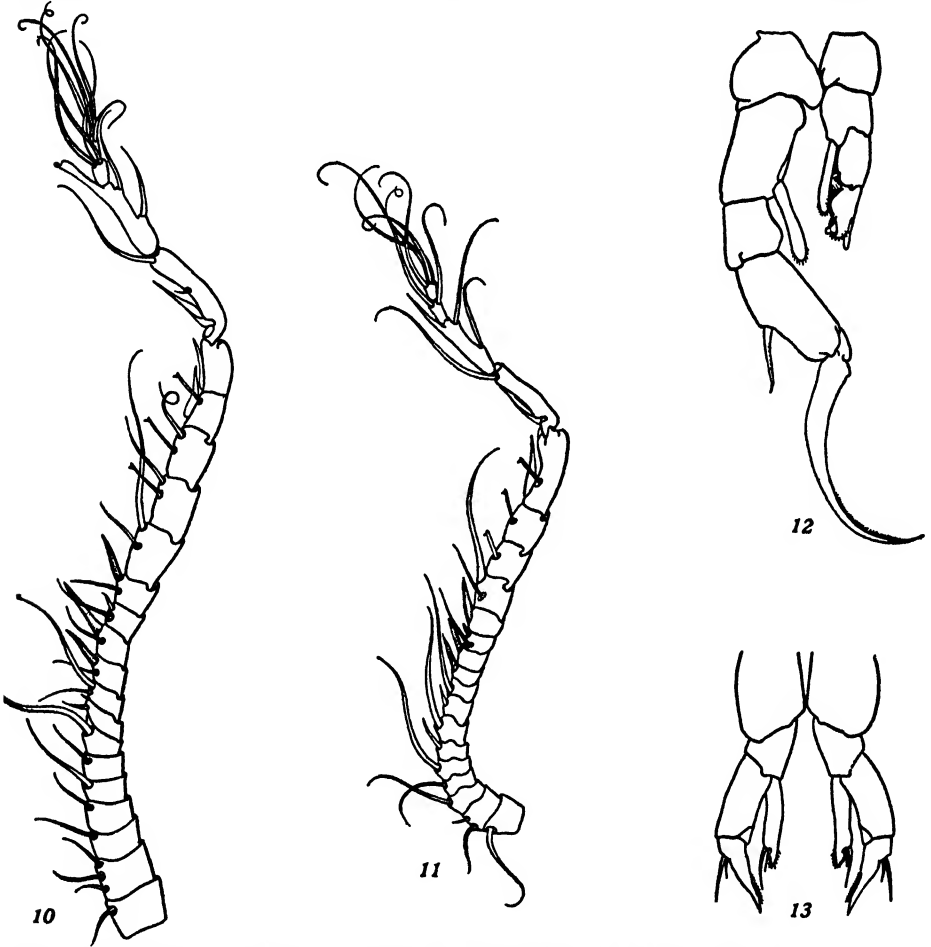


Fig. 10. Right antennule of male of *Diaptomus natriophilus* new species. ($\times 83$.)
 Fig. 11. Right antennule of male of *Diaptomus spinicornis* new species. ($\times 83$.)
 Fig. 12. Fifth legs of male of *D. spinicornis* in anterior view. ($\times 165$.)
 Fig. 13. Fifth legs of female of *D. spinicornis*. ($\times 165$.)

Diaptomus spinicornis new species
 (figs. 11-13 and 21)

Small, female about 1.30 mm. long, male about 1.10 mm. Antennules of female and left antennule of male delicate; setal formula as usual for the genus; small setae very slender, modified but little if at all. Right antennule of male (fig. 11) hardly swollen; major spines subequal in length, as long as, or slightly longer than, width of antennule below swollen portion, spine on segment 13 thickest; no spinous processes on swollen portion; depressed spinous processes as usual; process on antepenultimate segment long and very slender, tapering to a needlelike point and extending to or beyond middle of last segment.

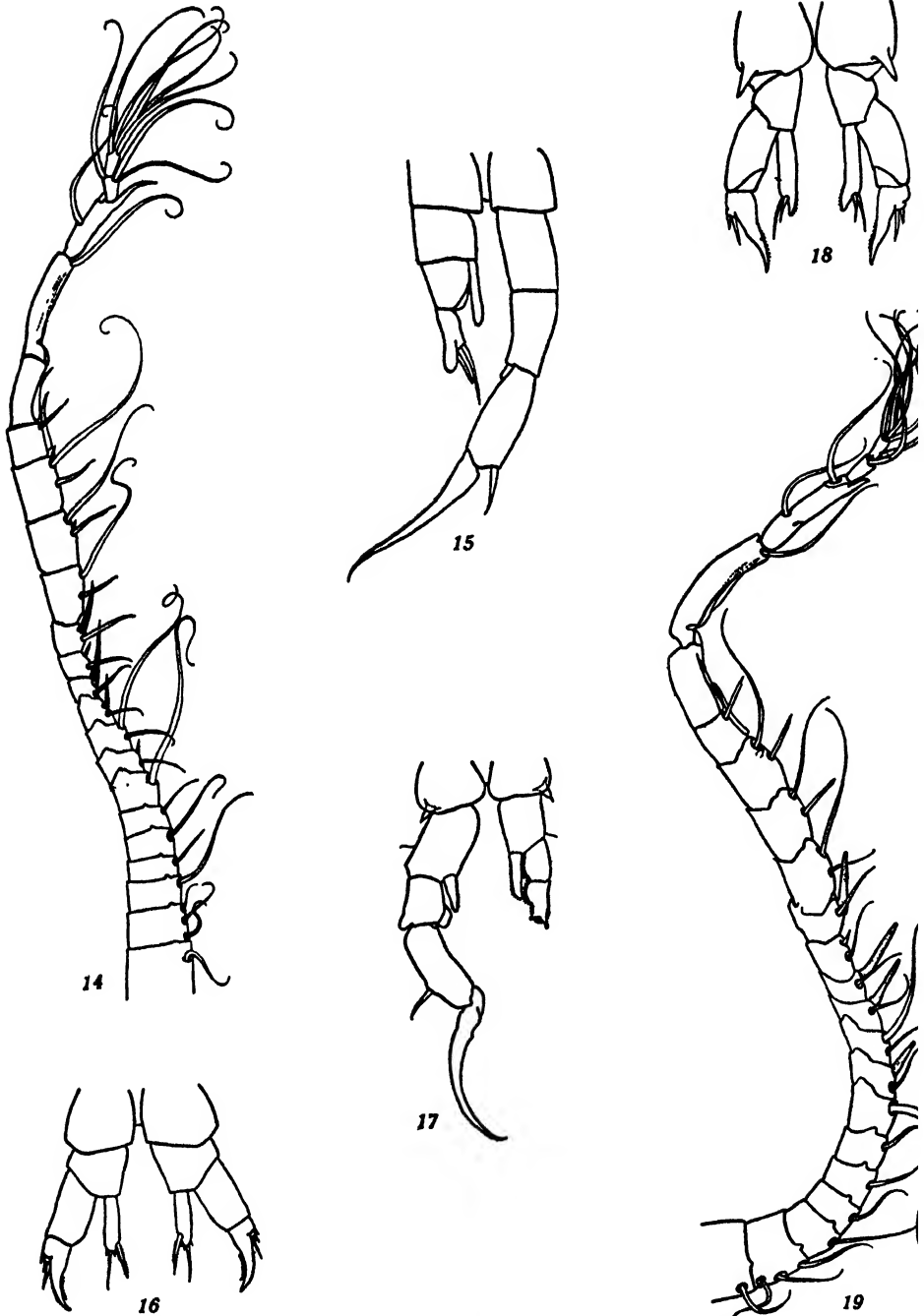


Fig. 14. Right antennule of male of *Diaptomus forbesi* new species. ($\times 85$.)

Fig. 15. Fifth legs of male of *D. forbesi* new species. ($\times 130$.)

Fig. 16. Fifth legs of female of *D. forbesi* new species. ($\times 105$.)

Fig. 17. Fifth legs of male of *Diaptomus connexus* new species. ($\times 100$.)

Fig. 18. Fifth legs of female of *D. connexus* new species. ($\times 125$.)

Fig. 19. Right antennule of male of *D. connexus* new species. ($\times 85$.)

Right fifth leg of male as in figure 12, left leg reaching to end of first segment of right exopodite; basal segment of right fifth leg with a bulbous lobe arising from anterior face of medial half and projecting between the two legs; second basal segment more than twice as long as wide, with a rounded enlargement on its proximal medial corner which opposes the inner lobe of the basal segment, otherwise nearly straight-sided, first segment of exopodite short, inner length less than outer, which is greater than width of segment; distal lateral corner continued as a rounded lobe; second segment of exopodite considerably more than twice as long as wide; inner margin straight, outer convex; spine situated slightly in front of middle of lateral margin, nearly as long as width of segment, curved in a distal and anterior direction in relation to the segment, slightly recurved near tip; distal margin serrate. Claw nearly as long as exopodite plus second basal segment, basal three-quarters thick, curved in general, distal quarter strongly curved and slender. Basal segment of left leg (fig. 12) with small, bluntly pointed lobe on anterior face lying beside larger lobe of right leg; second basal segment with convex medial surface and slightly convex lateral surface; distal segment of exopodite (fig. 21) as long as, or longer than, basal segment; depression between distal and proximal pads slight; both processes digitiform, relatively long and conspicuous, distal process longest, narrow, nearly three times as long as wide, somewhat laterally directed; proximal process narrow, blunt, directed medially and somewhat distally, with a group of several spinelets on its proximal face near distal end, as long as half the width of the process; spinelets on distal pad short, thick, medially directed; endopodites relatively long.

Fifth leg of female as in figure 13; narrowed apical portion of endopodites sharp distally, somewhat recurved at tip; apical setae unequal, smaller seta not much more than half as long as the longer; second segment of exopodite somewhat swollen, rounded, set off sharply from claw; seta of second segment obsolete; third segment obsolete, represented by a seta and a spine, seta twice as long as spine, reaching to middle of claw; claw thick in middle half, distally somewhat incurved; median half of inner margin with a line of 18-20 close-set spinelets (teeth), increasing in size distally; distalmost spinelet large and distally directed.

Syntypes.—U. S. Nat. Museum, no. 72571.

Taxonomic position.—The long, very slender process of the antepenultimate segment of the right antennule of the male, among other minor characters, distinguishes this species from all others of the *siciloides* group.

Distribution.—Taken by Professor Miller and Dr. Eakin in the same lake in Nevada, near Denio, Oregon, in which *Diaptomus nevadensis* was found.

Diaptomus connexus new species

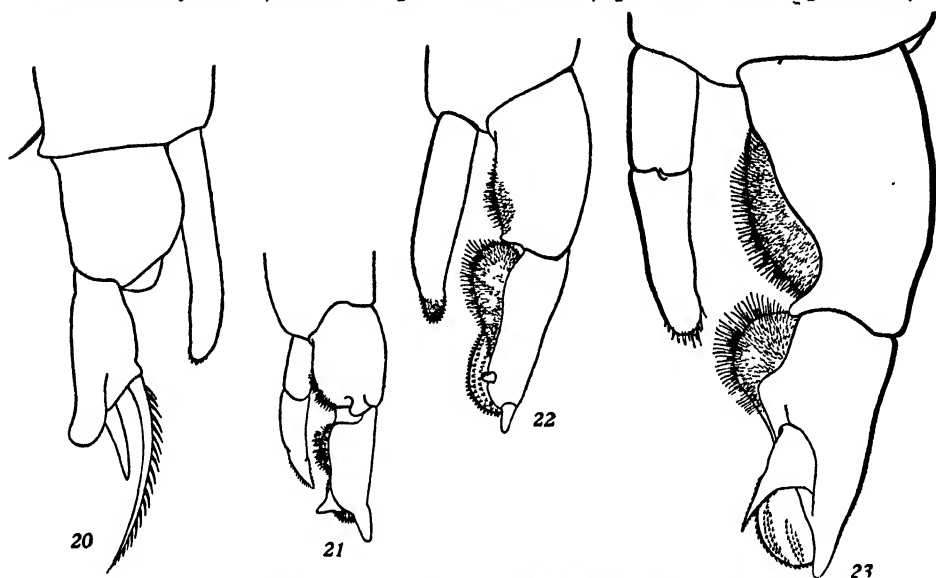
(figs. 17-19 and 22)

Females 1.30-1.75 mm. long, males 1.00-1.50 mm., exclusive of furcal setae. Metasome low and broad, tapering but little, posterolateral projections of female symmetrical, inconspicuous. Urosome slender, strongly asymmetrical in male; spines on genital segment of female minute.

Setal formula of antennules typical, three on segment 2, two each on 9, 22, and 23, five on 25, one on all others; outer seta on 23 vestigial. Right antennule of male (fig. 19) not conspicuously swollen; major spines on segments 8, 10, 11, and 13; spines slender, increasing in length distally, that on 8th segment not as long as diameter of segment, that on 10th slightly longer than diameter of segment, that on 11th about one-fourth longer, and that on 13th slightly (if at all) longer than that on 11th; swollen portion without spinous processes; spinous process on 23d segment about two-thirds as long as segment 24, slightly out-curved near tip.

Fifth legs of male (fig. 17) slender, left leg reaching about to distal end of first segment of right exopodite. First segment of right exopodite longer than broad, narrowest at base, its lateral distal margin produced as a rounded lobe and its inner margin bearing in distal half a rounded hyaline lamella (fig. 17) directed somewhat distally and arising from a

thick curved base. Second segment of exopodite about three times as long as wide, somewhat incurved, with convex outer and concave inner borders; spine somewhat in front of middle, shorter than diameter of segment, projecting at right angles to the segment, curved posteriorly; claw about as long as rest of exopodite. Right endopodite shorter than first segment of exopodite; left endopodite longer, reaching to middle of distal segment. Distal segment of left exopodite (fig. 22) nearly as long as basal segment, about half as wide as long; inner surface usually swollen, without conspicuous constriction; spinelets of anterior pad coarse,



Figs. 20-23. Exopodites, with endopodites, of left fifth legs of males of new species of *Diaptomus* drawn to scale from camera-lucida outlines. *D. forbesi* in posterior view, others in anterior view.

Fig. 20. *D. forbesi*

Fig. 22. *D. connexus*

Fig. 21. *D. spinicornis*

Fig. 23. *D. caducus*

hairs of posterior pad short and fine; appendages digitiform, terminal appendage longest, tapering; the other shorter, blunter, somewhat serrate on its proximal face, directed inward and distally.

Fifth legs of female (fig. 18) thicker and less elongated than in *D. tyrrelli*, third segment of exopodite obsolete, represented only by spine and seta; spine of second segment lacking; teeth of claw very slender and close-set. Apical setae of endopodite strikingly unequal.

Syntypes.—U. S. Nat. Museum, no. 72567.

Taxonomic position.—This species differs from all other species of the subgenus known to me, except *D. tyrrelli*, in the presence of a major instead of a minor spine on segment 8 of the right antennule of the male. It is easily distinguished from *D. tyrrelli*, chiefly by presence of a spinous process on the antepenultimate segment of the right antennule of the male, by the short, thick fifth legs of the female, which lack the spine of the second segment of the exopodite, and by the relatively small size of the major spine on segment 8 of the right antennule of the male.

Distribution.—Known only from Quail Lake (3350 feet), a shallow drainage lake on the southern slope of the Tehachapi Mountains in Kern County, California, where it was taken in early May in enormous numbers together with *D. franciscanus* and *Branchinecta*.

Subgenus *Aglaodiaptomus* new subgenus

Type species: *Diaptomus clavipes* Schacht, 1897.

Diagnosis.—Terminal segment of exopodite of left fifth leg of male (fig. 15) narrow, of about same width throughout; both processes long, subterminal, and distally directed (fig. 20); distal process a blunt spine, proximal process a long, straight or curving spine-like articulated seta, setose on its inner margin. Right fifth leg of male narrow and elongated; endopodite vestigial or obsolete; claw of exopodite short with but little curvature; spine of second segment of exopodite straight, relatively short, situated near distal end, directed distally and laterally. Fifth leg of female short and thick (fig. 16); claw of exopodite incurved, distinctly spinulose along both margins; third segment of exopodite very short but distinct; endopodite short with two long somewhat sinuous apical setae and smaller spinelike setae.

Major spines of right antennule of male (fig. 14) on segments 10, 11, and 13; a spinous process or hyaline lamella on penultimate segment.

The species listed below seem to belong in the new subgenus.

Diaptomus clavipes Schacht, 1897

D. conipedatus Marsh, 1907

D. leptopus Forbes, 1882

D. lintoni Forbes, 1893

D. forbesi new species

D. piscinae Forbes, 1893

D. stagnalis Forbes, 1882

Diaptomus forbesi new species
(figs. 14–16 and 20)

D. lintoni Marsh, 1929, from Laguna, California, nec *D. lintoni* Forbes, 1893.

Female 1.30 to 1.60 mm. long, and male 1.10 to 1.40 mm., exclusive of furcal setae. Metasome cylindrical, tapering posteriorly; posterolateral projections inconspicuous. Urosome short, slender, genital segment narrow, without lateral spines.

Antennules of female, and male right antennule, with five setae on terminal segment, three on segment 2, two on 9, 11, 16, 22, 24, and one on all others; minor spines on segments 8 and 12—as usual. Major spines on segments 10, 11, and 13 of right antennule of male (fig. 14); major spines very slender and sharp, that on segment 11 longest, that on segment 10 shortest; segment 15 with a very small spinelike spinous process just proximal to the specially modified seta, segment 16 with a small, distally located, strongly depressed spinous process; setae on segments 10–13 and posterior seta on segments 9 and 14 modified (fig. 14), stiff, somewhat sinuate, with recurved tips; process on segment 23 (fig. 14) longer than segment 24, tapering, distally outcurved.

Fifth legs of male (fig. 15) elongated, slender, left leg reaching to middle of second segment of exopodite of right leg; first segment of right exopodite twice its longest (basal) diameter, more than three-fourths as long as second segment; second segment narrowest basally, its outer margin convexly incurved in proximal half; spine near distal end, directed distally and somewhat laterally, about as long as maximum diameter of segment; claw heavy at base but very slender in distal half, finely serrate, as long as rest of exopodite, or nearly so. Right endopodite obsolete, left reaching to about middle of terminal segment. Two segments of left exopodite (fig. 20) subequal in length; terminal segment narrower, contracted basally, apparently lacking spinelets or hairs, tip extending distally as a blunt lobe considerably beyond base of distal process; distal process spinelike, directed distally and medially; proximal process a long, stiff pinnate seta; both processes borne on an oblique chitinized plate.

Fifth legs of female (fig. 16) short and thick; third segment of exopodite very small but distinct, seta twice as long as spine; spine on second segment small, hardly reaching beyond third segment; claw sharply curved, toothed along both faces; endopodites about as long as basal segment of exopodite, without apical extension, bearing a number of short spinelets and two pinnate apical setae, about two-thirds as long as endopodite.

Syntypes.—U. S. Nat. Museum, no. 72568.

Taxonomic position.—*D. forbesi* is most closely related to *D. lintani* Forbes. Indeed, Marsh (1929) evidently confused the two since he reports *D. lintoni* from the lake from which my material was taken. The present species differs most strikingly from *D. lintoni*, as described and figured by Forbes (1893), in that (1) the process on the antepenultimate segment of the right male antennule is much larger, reaching appreciably beyond segment 24 (fig. 14), (2) segment 2 of the right fifth exopodite of the male is relatively shorter and broader, and (3) the claw is nearly as long as the rest of the exopodite (shorter than last segment in *D. lintoni*!).

Distribution.—Known only from collections kindly made for me, in June, from two small, contiguous lakes in Laguna Canyon, Orange County, California, by Mr. Arthur B. Burch, of the department of Zoölogy, University of California.

**BRACKISH AND FRESH-WATER NEREIDAE
FROM THE NORTHEAST PACIFIC, WITH
THE DESCRIPTION OF A NEW SPECIES
FROM CENTRAL CALIFORNIA**

BY

OLGA HARTMAN

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THE FAMILY NEREIDAE is unique among the polychaetous annelids in that it includes many (almost 50) euryhaline species. These species belong to 11 genera; about half of them are representatives of the genus *Namanceris* Chamberlin (= *Lycastis* of most authors) or the closely related genera *Lycastoides* Johnson and *Lycastopsis* Augener. Furthermore, over half of them are known only from tropical or subtropical waters of the western Pacific.

The northeast Pacific is known to have but few nereids except in strictly marine habitats. It seems likely, however, that the numerous drainages along coastal California contain additional species. The cosmopolitan *Neanthes succinea* (Frey and Leuckart) abounds in the brackish waters of Lake Merritt in Oakland, California, as well as in San Francisco Bay and its estuaries. *Neanthes saltoni* Hartman was recently (1936) described from Salton Sea, California, a twentieth-century revival of the former (1853) extensive Cahuilla Lake. The history of *N. saltoni* is an intriguing problem. That the nereid is a survival of marine invasion is hardly likely, since Cahuilla Lake is said to have dried up by the late nineteenth century, depositing great quantities of salt in the depression which subsequently became Salton Sea. There is, however, an unconfirmed report that a small channel of salt water persisted during this period. Later inundation by the fresh waters of the Colorado River established the present lake. Since the floodwaters of the Colorado overflowed into the lake several times in the course of the twentieth century, its salinity must have fluctuated materially.

Two strictly fresh-water nereids are *Lycastoides alticola* Johnson (1903, p. 212), from a 7000-foot mountain stream, Sierra Laguna, Lower California, and *Nereis limnicola* Johnson (1903, p. 208), from Lake Merced, San Francisco, California. *N. limnicola* has not been recovered since first described. Merced is a fresh-water lake, separated from the Pacific Ocean by a narrow strip of sand dunes. It is thought to have been an arm of the sea in late Quaternary times (Lawson, 1895; Johnson, 1903). During the past three decades or more the lake has been part of the water system of the city of San Francisco. Its boundaries and bed have been appreciably altered by dredging and road-building operations, and what was once the type locality of *N. limnicola* now lies many feet below a road bed.

In the summer of 1935 a new species of the genus *Neanthes* Kinberg was found inhabiting the sandy mud banks of tidal streams in Marin and Sonoma counties, California. I take pleasure in naming the species *N. lighti* sp. nov.

for Professor S. F. Light, who made possible the original discoveries. The specimens were taken a few miles inland from the mouths of the streams, associated with a spionid (*Boccardia brachycephala* Hartman), the shore crab (*Hemigrapsus oregonensis* Dana), and hemipterous insects. More recently the same species was taken by Mrs. A. R. Grant from small fresh-water pools along the banks of the Russian River, near Healdsburg, California, associated there with the ammocoetes larvae of *Petromyzon* and with chironomid larvae. *N. lighti* is thus clearly adjusted to brackish and fresh water.



Figs. 1-4. Camera lucida drawings of *Neanthes lighti* sp. nov.

Fig. 1. Tenth parapodium in anterior view. $\times 45$.

Fig. 2. Median parapodium in posterior view. $\times 45$.

Fig. 3. Tenth from the last parapodium in posterior view. $\times 45$.

Fig. 4. Ventral neuroseta from a posterior parapodium. $\times 645$.

A KEY TO THE SPECIES OF NEANTHES KINBERG FROM CALIFORNIA

1. Median and posterior dorsal cirri with base of attachment on distal half of dorsal edge of dorsal lobe. 2
1. Median and posterior dorsal cirri with base of attachment on proximal half of dorsal edge of dorsal lobe (fig. 2) 3
2. Larger; marine; areas v to VIII of proboscis forming a continuous band on oral ring; posterior dorsal cirri inserted at middle of dorsal edge of lobe.
N. brandti (Malmgren), 1866 (= *Alitta plenidentata* Moore)
2. Smaller; brackish or estuarine; areas v to VIII not continuous as a band; posterior dorsal cirri inserted at ends of dorsal lobes. *N. succinea* (Frey and Leuckart), 1847
3. Area v with paragnaths; posterior parapodia with foliaceous dorsal lobe and trapezoidal median lobes. *N. saltoni* Hartman, 1936
3. Area v without paragnaths; posterior parapodia with triangular dorsal and median lobes (fig. 3) *N. lighti*, p.

Neanthes lighti sp. nov.

(figs. 1-4)

Diagnosis.—Length 25-45 mm. (Russian River specimen); width 2.5-4 mm. without, and 4-7 mm. with parapodia, broadest at segments 7-10. Number of segments 45-82. Prostomium broader than long, with a bluntly rounded anterior margin and a shallow, longitudinal median depression in anterior half. Antennae inserted on middle third of free anterior mar-

gin, separated at base by half their length; antennae extend distally to beyond middle of palpophore. Eyes dark, widely separated. Palpi stout, dorsally somewhat wrinkled; palpophores about as long as prostomium; palpostyles tiny, hemispherical.

Peristomium only slightly longer than segment 2, wrinkled on lateral and ventral sides. Peristomial cirri tapering, the longest reaching distally to segment 7, the shortest about as long as prostomium.

Paragnaths black, tiny, those of oral ring notably smaller than those of maxillary ring; teeth of area II the largest. Area I with a single cone; II with 12-14 tall cones in a wide-open crescent; III with 20-25 points in a broad patch separated from area IV by a space; IV with 30-35 cones in a crescent; V with none; VI with 3 tiny points disposed in a triangle; VII-VIII a continuous row of about 20 tiny points, a few of these on ventral side forming a short second row.

Parapodia well developed throughout, largest in anterior region. Anterior parapodium with dorsal, median, and ventral lobes, as shown in figure 1; acicular lobes elongate, exceeding in length those of more posterior parapodia. Median parapodium as shown in figure 2, differing from anterior parapodium chiefly in having a stouter base and more attenuate dorsal and ventral lobes. Posterior parapodium (40th) as shown in figure 3; median lobe greatly reduced, ventral lobe exceeded distally by neuroacicular lobes, the latter without free portions.

Notopodia of anterior region each provided with about 10 spinigers; neuropodia with about 7 spinigers and 5 falcigers in superior part of fascicle, and about 7 spinigers and 7 falcigers inferiorly (fig. 4). Setae of posterior parapodia disposed similarly, but reduced; notopodia with about 4 spinigers, neuropodia with about 1 spiniger and 2 falcigers superiorly and with 3 spinigers and 8 falcigers inferiorly.

Anal cirri 2, styliform, as long as last 7 segments.

Color in life pale, translucent; in preservative, dusky olive in anterior third, deepest on prostomium and first 5 segments.

Type specimen in the United States National Museum.

Distribution.—Tomaes Creek, Marin County, California (type), Stempell Creek, southern Sonoma County, California; small fresh-water pools along the Russian River, near Healdsburg, California (collected by Mrs. A. R. Grant). Inhabits thin, pale brown, loosely constructed tubes in vertical burrows in sand and clay-sand banks.

Discussion.—*Neanthes lighti* resembles *N. sakhalinensis* (Okuda) described in 1935 from brackish lakes near Sakhalin, Japan. The two species are similar in body size and prostomial proportions. *N. lighti* differs from the Japanese species in its dentition, notably the smaller teeth of the oral ring, in the nature of the dorsal and middle lobes of the parapodia, especially in the posterior region, and in the insertion of the dorsal cirri. In *N. lighti* these are inserted near the mid-dorsal point of the dorsal lobe (fig. 3), and the neurosetae have longer appendages (fig. 4).

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THE ANATOMY OF THE GASTEROPOD CREPIDULA ADUNCA SOWERBY

BY
C. E. MORITZ

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INTRODUCTION

THE GASTEROPOD *Crepidula adunca* Sowerby is a protandrous pectinibranch living on the black turban shell *Tegula funebris* (A. Adams), the range of the two being the same, from Vancouver, British Columbia, to the tip of Lower California. In central and northern California *Tegula funebris* is abundant on protected, rocky shores, and hence *Crepidula adunca* is easily obtained. *C. adunca* breeds throughout the year, has a large egg, favorable for embryological investigations, and retains its young beneath the shell until they reach young adulthood. This paper describes the anatomy of the young male, the study having been undertaken because no complete account was available for any species of the genus. The bibliography contains references to such fragmentary contributions as have been made to the anatomy of the genus *Crepidula*.

For assistance in bringing this investigation to completion, the writer is much indebted to Professor S. F. Light, of the University of California.

MATERIAL AND TECHNIQUE

The animals used were obtained at Jenner, Sonoma County, California. They were fixed in Zenker's, Kleinenberg's, or corrosive sublimate fixatives, with acetic acid. The expanded condition was best retained by pouring the boiling fixative on the animals, but even this method was not wholly satisfactory.

Before sectioning, the specimens were put in diaphenol for twelve hours to soften the radula. They were then imbedded in paraffin, sectioned at 10μ , and stained with Delafield's hematoxylin and eosin. Celloidin sections of 200μ , stained in alum carmine, were helpful in determining the relative positions of organs. Wall's method (1932) was followed in making celloidin sections.

ANATOMY OF THE ADULT MALE

External features.—The adult shell of *Crepidula adunca* is limpetlike, with the apex markedly peaked and directed backward (fig. 2). Internally the shell bears a nacreous shelf. The visceral mass of the animal lies above the shelf, occupying the peak of the shell (fig. 1, *vh*). The posterior end of the foot, or the metapodium, lies between the shelf and the substrate. The shell is nearly bilaterally symmetrical; the apex, however, veers slightly to the right. This is the only indication in the adult shell of the asymmetrical condition resulting from torsion.

The mantle (fig. 1) is in contact with the entire inner surface of the shell and extends over the head and ctenidium like a hood.

The foot (figs. 1, 6) is divisible into three regions: (1) the propodium, or forward extension of the foot, (2) the mesopodium, or the region connected to the body proper, and (3) the metapodium, or posterior extension of the foot.

The head is provided with two blunt tentacles. In the male a proboscislike penis (fig. 6, *p*) is attached to the neck immediately behind the right tentacle. Along the lateral borders of the neck lie two broad, flat sheets, the neck lappets (fig. 3, *nl*). Above the head and neck and beneath the mantle fold are rodlike projections, which are the filaments of the ctenidium. A few of these filaments are shown in figure 5. Slightly to the left of, and dorsal to, the head, the mantle is folded into a pocket, the food pouch (fig. 3, *fp*).

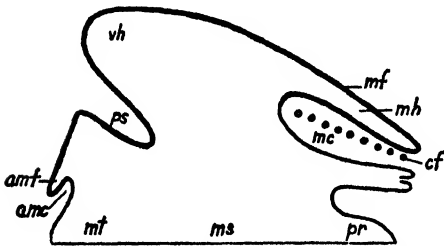


Fig. 1. Sagittal section of figures 3–6. The mantle is shown in heavy black line.

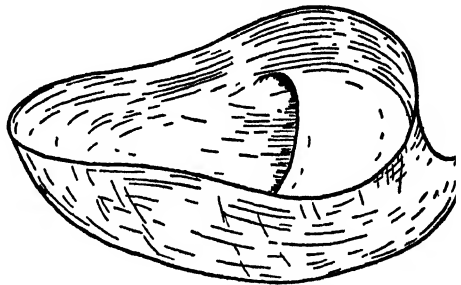


Fig. 2. The shell of an adult *Crepidula adunca* from a lateral and ventral view. $\times 7$.

amc, accessory mantle cavity; *amf*, accessory mantle fold; *cf*, ctenidial filament; *mc*, mantle cavity; *mf*, mantle fold; *mh*, mantle hemocoel; *ms*, mesopodium; *mt*, metapodium; *pr*, propodium; *ps*, position of the shell shelf; *vh*, visceral hump.

Alimentary tract and associated organs (fig. 3).—Four organs not actually a part of the alimentary tract are concerned with procuring food. They lie within the mantle cavity and are the ctenidium (fig. 5, *cf*), the endostyle (fig. 3, *en*), the food pouch, and the ciliated groove of the right neck lappet. The endostyle is a ridge of mucus-secreting cells, extending from the posterior left-hand corner of the mantle cavity obliquely downward and forward along the left wall of the mantle cavity to the anterior end of the mantle fold (fig. 3, *en*). The ridge lies immediately below the ctenidial filaments. No other gastropod is known to possess such an organ. Orton (1912b, pp. 472–473), however, believes it probable that all Calyptraeidae and possibly other sedentary gastropods, such as the Hipponycidae and Capulidae, may feed in a manner similar to *Crepidula*. Hence a comparable organ which is homologous, analogous, or both, to an endostyle, may be present in those forms.

Along the dorsal face of the right neck lappet there are two ciliated grooves, (1) a wide, shallow one, the food groove, median to (2) a much smaller and deeper groove, which is used for sperm transfer (fig. 3, *fg*, *sp*). The mouth leads into the pharynx, into which the two salivary glands and the radular sac open. From the pharynx the oesophagus runs posteriorly and, passing between the anterior and posterior diverticula of the digestive gland, enters the posterior end of the pear-shaped stomach (fig. 3, *st*).

The stomach is lined with a secreted material (Berkeley, 1935), whereas

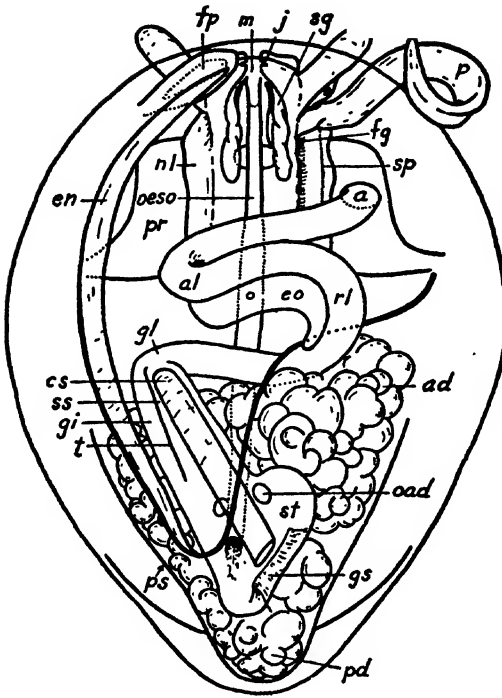


Fig. 3. Diagram of the adult digestive system and the associated organs as seen from above. The heavy black line outlines the mantle cavity. $\times 25$.

a, anus; *ad*, anterior diverticulum of the digestive gland; *al*, anal loop; *cs*, crystalline style; *en*, endostyle; *eo*, excretory organ; *oeso*, oesophagus; *fg*, food groove; *fp*, food pouch; *gi*, gastric end of the intestine; *gl*, gastric loop; *gs*, gastric shield; *j*, jaw; *m*, mouth; *nl*, neck lappet; *oad*, orifice of the anterior diverticulum into the stomach; *p*, penis; *pd*, posterior diverticulum; *pr*, propodium; *ps*, position of the shell shelf; *rl*, renal loop; *sg*, salivary gland; *sp*, sperm groove; *ss*, style sac; *st*, stomach; *t*, typhlosoles.

the oesophagus and intestine are lined with ciliated epithelium. On the posterior and right walls of the stomach this hard lining forms a bladelike ridge, the gastric shield (fig. 3, *gs*), against which the crystalline style rubs (Mackintosh, 1925). There are two diverticula of the digestive gland. They compose a large portion of the visceral mass. Each opens separately into the stomach on the ventral floor of the latter organ, as indicated in figure 3, *oad*.

To the left the stomach opens into a wide tube which is incompletely divided into two halves by the major and minor typhlosoles (Mackintosh, 1925, pl. 20, fig. 3). The median half is the style sac, lined with powerful cilia which rotate the crystalline style and drive it rearward onto the gastric shield. The lateral half is the gastric end of the intestine. The anterior end of this dual tube extends beneath the floor of the mantle cavity in the adult, but not in a newly hatched individual. Anteriorly the style sac and the gastric end of the intestine merge into one tube (fig. 3), which curves sharply to the right as the gastric loop of the intestine and runs slightly posterior and to the right side of the body, where it curves upward and around the posterior wall of the mantle cavity (fig. 3) to lie in the mantle fold. A second loop, the renal loop, carries the intestine again to the left, skirting the excretory organ. A third loop, the anal loop, curves to the right, in which direction the intestine runs, to end at the anus on the right mantle wall above, and slightly to the right of, the neck.

Nervous system.—The general features are indicated in figure 4. The fusion of the suboesophageal ganglion to the right pleural ganglion and the presence of the propodial ganglia, not shown in figure 4, are noteworthy. The propodial ganglia, readily seen in transverse sections of adults or in developing embryos, have not been reported previously. They lie immediately ventral to the pedal ganglia. One other important item of the nervous system is the marginal mantle nerve, which runs in the mantle hemocoel around the periphery of the body, as shown in figure 4. The delicate commissure shown by Heath (1916, p. 481) ventral to the oesophagus and uniting the two cerebrobuccal connectives, could not be found. Heath (1916) gives a more detailed account of the nervous system.

Sense organs (fig. 4).—Near the base of each tentacle an eye is situated beneath the external epithelium of the integument. On the left mantle wall (fig. 4, *o*) below the endostyle lies the osphradium with its six to eight blunt projections. Posterior to all the ganglia lie two statocysts (fig. 4, *sc*), each with a single large statolith. The pedal ganglia innervate the statocysts.

Excretory system (figs. 3, 5).—The excretory organ lies in the hemocoel of the mantle fold above the base of the neck and within the renal loop of the intestine. It opens into the mantle cavity by a ciliated pore just above the brain region; there is no duct. Nor could a renopericardial pore be found, although it may be present.

Respiratory system (figs. 1, 5).—The respiratory organs of the adult are the ctenidium and portions of the mantle. The ctenidium (fig. 5, *cf*) consists of 50 to 100 filaments projecting forward and to the right from the dorsal side of the endostyle. The mantle is shown by the heavy black line in figures 1 and 3.

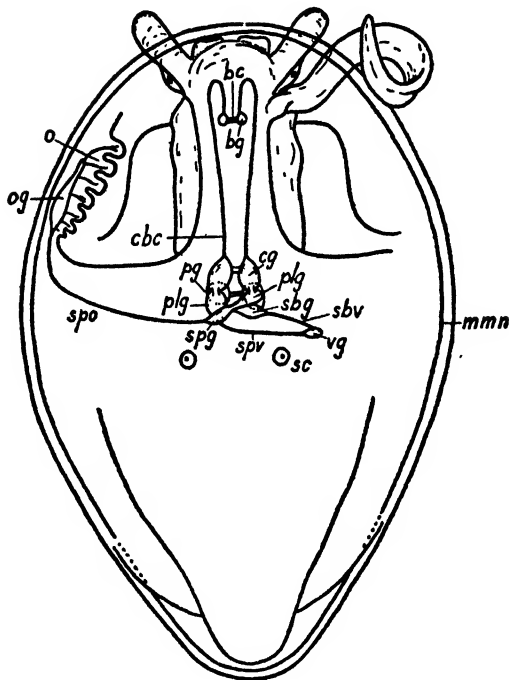


Fig. 4. Diagram of the nervous system and sense organs of the adult as seen from above. The innervation of the statocysts from the pedal ganglia is not shown, nor are the several connections between the pleural ganglia and the marginal mantle nerve. $\times 25$.

bc, buccal commissure; bg, buccal ganglion; cbc, cerebrobuccal connective; cg, cerebral ganglion; mmn, marginal mantle nerve; o, osphradium; og, osphradial ganglion; pg, pedal ganglion; plg, pleural ganglion; sbg, suboesophageal ganglion; sbv, suboesophageal-visceral connective; sc, statocyst; spg, supraoesophageal ganglion; spo, supraoesophageal-osphradial connective; spv, supraoesophageal-visceral connective; vg, visceral ganglion.

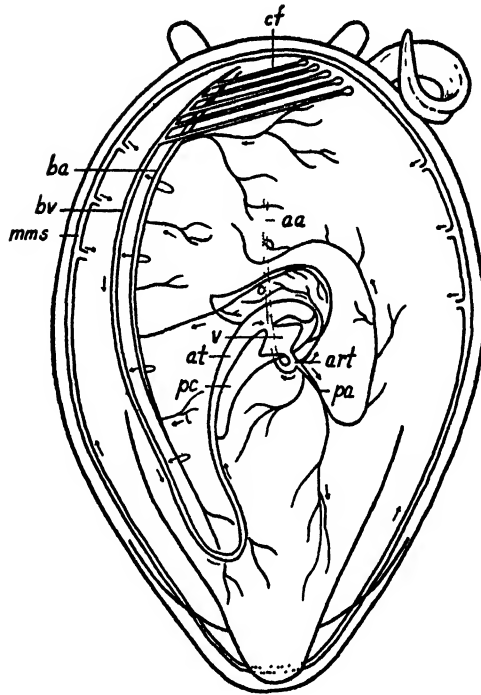


Fig. 5. Diagram of the blood and respiratory systems of the adult as seen from above. Only five of the many ctenidial filaments are shown. The anterior artery, indicated by the dotted lines, is the only blood vessel which does not lie in the mantle; it lies ventral to the floor of the mantle cavity. The recurved arrows shown between the branchial artery and vein represent the flow of the blood into the ctenidial filaments and out. The sinuses are not indicated. $\times 25$.

aa, anterior artery; *art*, arterial trunk; *at*, atrium; *ba*, branchial artery; *bv*, branchial vein; *cf*, ctenidial filaments; *mms*, marginal mantle sinus; *pa*, posterior artery; *pc*, pericardial cavity; *v*, ventricle.

Over the mantle cavity it extends as the mantle fold (fig. 1, *mf*), containing the mantle-fold hemocoel, in which lie the intestine, heart, and excretory organ. Laterally, the mantle cavity proper continues around the periphery of the mesopodium and metapodium as the accessory mantle cavity (fig. 1, *amc*), and the fold forming this cavity is the accessory mantle fold.

Blood vascular system.—The blood vascular system is shown in figure 5. It agrees with the description given by Kleinstaubert (1913) for the closely allied genera, *Trochita*, *Calyptraea*, and *Janacus*.

Muscular system (fig. 6).—Muscles of the adult may be classified into the pedal, the buccal, and the dermal groups. Pedal muscles may be separated into several subgroups according to origins and insertions. Muscle fibers in the propodium and metapodium run dorsoventrally and are the propodial and metapodial muscle masses, respectively. In the mesopodium the fibers are fixed at the ventral end to the epithelium of the foot and at the dorsal end to the shell and its shelf. The two groups of fibers running to the sides of the shell (fig. 6, *shm*) constitute the shell muscles. The sheet of muscle fibers fixed to the shell shelf (fig. 6, *sfm*) constitutes the shelf muscle.

The buccal group of muscles consists of the protractors and retractors of the tongue and radula. The dermal muscles are the more or less isolated fibers attached at each end to the external epithelium. They contract the mantle, the tentacles, and the head.

Reproductive system (fig. 6).—The gonad in the male lies under the floor of the mantle cavity. On the right side a tube, the vas deferens, leads from the testis to the right posterior corner of the mantle. Here a diagonal external ciliated groove runs anteriorly and to the left, continuing forward on the right neck lappet (fig. 6, *sp*), thence to the ventral surface of the penis, almost to its tip.

Details of structure of the reproductive organs have been given by Giese (1915) for *Crepidula unguiformis*, *Calyptraea*, and *Capulus*. The metamorphosis of the gonad from the male to the female condition has been worked out in great detail by Gould (1917).

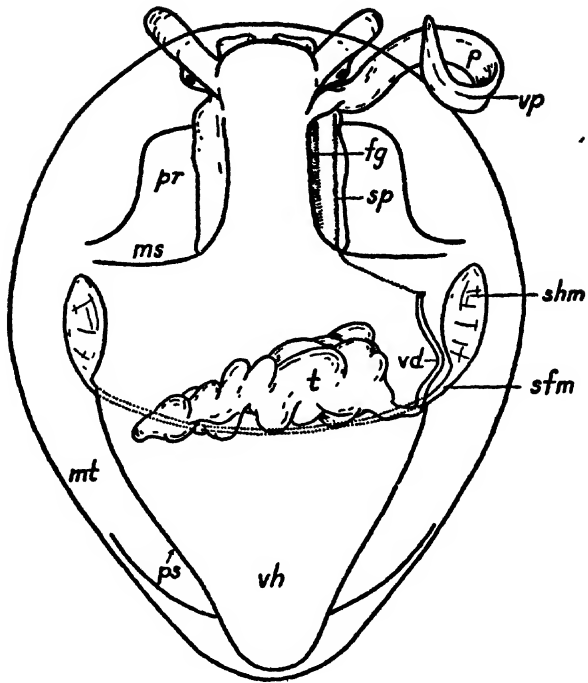


Fig. 6. Diagram of the reproductive and muscular systems of the adult as seen from above. Only the areas of attachment of the shell and shelf muscles are shown. $\times 25$.

fg, food groove; *ms*, mesopodium; *mt*, metapodium; *p*, penis; *pr*, propodium; *ps*, position of the shell shelf; *sfm*, shelf muscle; *shm*, shell muscle; *sp*, sperm groove; *t*, testis; *vd*, vas deferens; *vh*, visceral hump; *vp*, ventral groove of the penis.

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**DESCRIPTIONS OF NEW SPECIES AND
NEW GENERIC RECORDS OF
POLYCHAETOUS ANNELIDS FROM
CALIFORNIA OF THE FAMILIES
GLYCERIDAE, EUNICIDAE,
STAURONEREIDAE AND OPHELIIDAE**

**BY
OLGA HARTMAN**

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DESCRIPTIONS OF NEW SPECIES AND NEW GENERIC RECORDS OF POLYCHAETOUS ANNELIDS FROM CALIFORNIA OF THE FAMILIES GLYCERIDAE, EUNICIDAE, STAURONEREIDAE, AND OPHELIIDAE

BY
OLGA HARTMAN

THE FAMILY Glyceridae, as distinct from the family Goniadidae, is represented in California by two genera, *Glycera* Savigny and *Hemipodus* Quatrefages. These genera are separable as follows:

Parapodia biramous; setae simple and composite.....*Glycera* Savigny
Parapodia uniramous; setae composite only.....*Hemipodus* (see below)

Genus *Hemipodus* Quatrefages

Key to the Species of *Hemipodus* from California

Parapodia elongate (fig. 1), as long as, or longer than, their respective segments;
everted proboscis clavate; smaller, less than 20 cm. long....*H. borealis* Johnson, p. 94
Parapodia much shorter than their respective segments (figs. 2-4); everted proboscis
almost cylindrical; larger, up to 25 cm. long.....*H. californiensis* sp. nov., p. 93

Hemipodus californiensis sp. nov.

(Figs. 2-7)

Diagnosis.—Long, slender, terete, length up to 25 cm., width up to 3.5 mm. with, 3.0 mm. without, parapodia; number of segments 170-220. Anterior segments biannulate, posterior segments faintly triannulate, the rings indicated by faint transverse lines. Segments more than half as long as wide.

Prostomium slender, smooth, indistinctly 7- or 8-ringed, almost 3 times as high as wide at base; tipped with 4 minute, equal, cirriform tentacles. Proboscis greatly elongate when everted, subcylindrical, tapering slightly caudad. Papillae of proboscis of one kind, clavate, elongate (fig. 7), distributed diffusely over proboscis except at the base, where about 20 longitudinal rows are distinguishable. Jaws 4, black, held between segments 25 and 26 when the proboscis is retracted and posterior to head when the proboscis is everted. Each jaw piece (fig. 6) with a free portion which is somewhat shorter than the embedded portion. Aileron a straight rod (fig. 6), almost at right angles to the main axis of the jaw.

Parapodia reduced, slightly ventrolateral in anterior and median regions, but gradually acquiring a strictly lateral position in posterior region; varying little throughout length although somewhat more slender and elongate in posterior region. Presetal lobe much the larger, extending ectad almost to middle of free length of setae (fig. 3); postsetal lobe slightly pointed but short throughout (figs. 2-4). Dorsal and ventral cirri small, papillar, situated above and below bases of the parapodia, respectively (figs. 2-4). Setae slightly heterogomph (fig. 5), 16-24 in a simple, fan-shaped fascicle, about equally distributed above and below the aciculum. Aciculum straight, tapering, occurring singly in parapodia.

Color in life satiny, light green; in alcohol, pale salmon or tan.

Holotype.—U. S. Nat. Mus. no. 20361.

Distribution.—Morro Bay (type), Elkhorn Slough, Balboa Bay, California. Inhabits sandy mud flats.

Discussion.—*H. californiensis* differs from *H. borealis* Johnson in being larger and more elongate; in life it is green instead of reddish. Its parapodial lobes are shaped otherwise (figs. 1–4); the proboscis is covered with clavate papillae.

Hemipodus roseus (Blainville) (*vide* Quatrefages, 1865; p. 194) and *Hemipodia patagonica* Kinberg (1857–1910) may possibly be identical with *H. bo-*



Fig. 1. *Hemipodus borealis*. Anterior parapodium in anterior view. Setae omitted. $\times 17$.

Figs. 2–7. *Hemipodus californiensis*

Fig. 2. Anterior parapodium in posterior view. Setae omitted. $\times 17$.

Fig. 3. Median parapodium in posterior view. Setae diagrammatically represented. $\times 17$.

Fig. 4. A midposterior parapodium in posterior view. Setae omitted. $\times 17$.

Fig. 5. Articulation of composite seta. $\times 290$.

Fig. 6. Jaw and attached aileron. $\times 67$.

Fig. 7. Papillae of proboscis. $\times 126$.

Figs. 8, 9. *Eunice biannulata*

Fig. 8. Fifteenth parapodium from MCZ type of *Leodice valens*. $\times 10$.

Fig. 9. One-hundredth parapodium from same. $\times 10$.

Figs. 10, 11. *Eunice enteles*

Fig. 10. Fifteenth parapodium from MCZ type of *Leodice enteles*. $\times 21$.

Fig. 11. One-hundredth parapodium from same. $\times 21$.

realis Johnson (1901). The first was described from Chile, the second from the Straits of Magellan; *H. borealis* has been reported from western Canada (Berkeley, 1927, p. 411) south to San Diego, California (Moore, 1909, p. 259). I have seen no representatives from South America.

Family EUNICIDAE

Much discussion has centered about the status of the name *Eunice*, type of the family Eunicidae. European students have generally adopted the name *Eu-*

nice Cuvier (1817). Some American authors have given reasons why *Leodice* Savigny (1820) should be used. Verrill (1900, p. 638) thought that Cuvier's use of *Eunice* had been antedated by that of Hübner in 1816 for Lepidoptera. Hübner, however, used the name *Eunica*, and it was only in 1832 that C. Geyer incorrectly used *Eunice* for *Eunica* (vide Schultz-Kükenthal-Heider, Generic, Subgeneric Record, *E*). Treadwell (1921, 1922) in his extensive studies on this family has followed Verrill's use of *Leodice*.

Another name should be mentioned in recalling the history of the type genus of this family. In 1816, Lamarek described and figured two chitinous tubes of what he called polyps, erecting the genus *Tibiana*, with species *T. ramosa* and *T. fasciculata*, and with type locality New Holland. There seems no reason to doubt that the characteristic tubes which he described are those of the genus under discussion. However, in view of the wide acceptance of the name *Eunice* and the obscurity surrounding the name *Tibiana* Lamarek, I am retaining the former.

The family Eunicidae has been recorded from California through three genera, *Eunice* Cuvier, *Marphysa* Quatrefages, and *Eriphyle* Moore (non Kinberg). *Eunice* (*Eriphyle*) *paloloides* Moore belongs with those eunicids in which the branchial structures are absent in anterior segments, in which certain setae characteristic of *Eunice* are absent, and in which the structures of the proboscis differ (see below). The name *Eriphyle* Kinberg cannot be used for this group of eunicids since it was first applied to *E. capensis* Kinberg, a typical *Eunice*. A species of the *paloloides* group was described by Gray, in 1847, under the name *Palolo viridis*. Subsequent authors have included *P. viridis* in the genus *Eunice*. I suggest retaining the genus *Palolo* for this species as well as those which are closely related and which may be characterized as outlined below (p. 98). Two species of *Palolo* are represented in California, *P. paloloides* (Moore) and *P. pallidus* sp. nov. (p. 99).

Species in two other genera are hereby reported from California, *Lysidice ninetta* Audouin and M. Edwards, from Laguna Beach, Orange County, and *Nematonereis*, probably *N. unicornis* (Grube), from Moss Beach, San Mateo County. The latter is represented only by a tiny (4 mm. long) individual with four eyespots, as described by Ehlers for *N. oculata* (Ehlers, 1868, p. 314).

Genus *Eunice* Cuvier, 1817

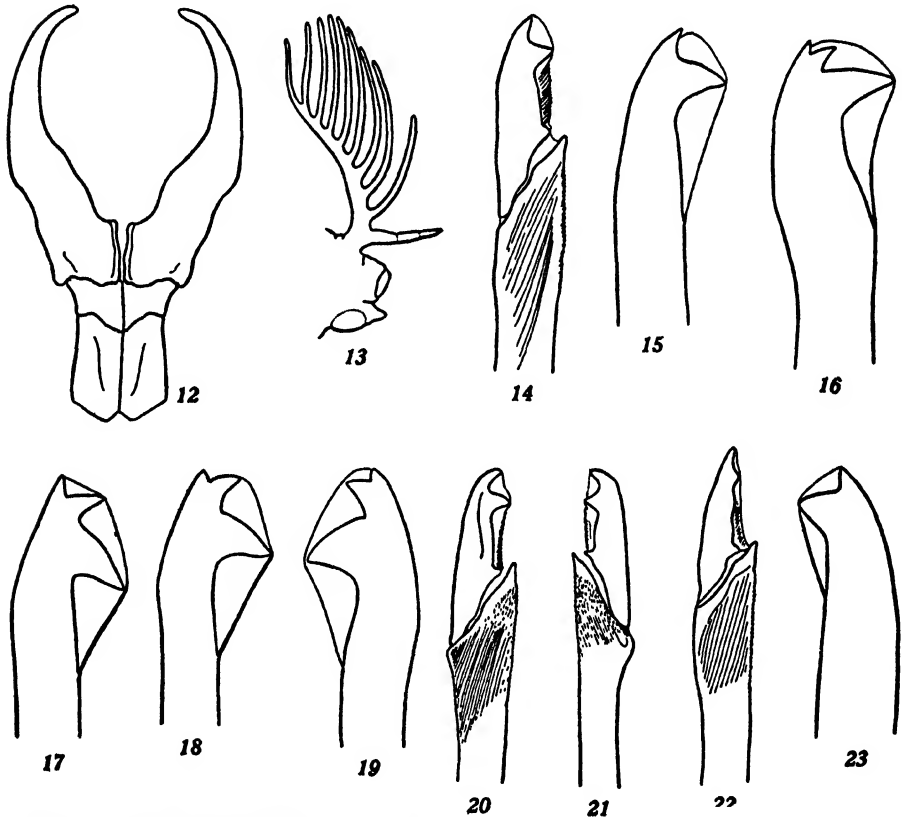
Includes *Leodice* Savigny and *Eriphyle* Kinberg (see above); non *Palolo* Gray.

Discussion.—Through the courtesy of the Museum of Comparative Zoölogy at Harvard it has been possible to study typical parapodia of the holotypes of *Leodice enteles* Chamberlin (figs. 8, 9) and *L. valens* Chamberlin (figs. 10, 11). Both of these had been given only preliminary treatment (Chamberlin, 1918, 1919b). Through the kindness of the United States National Museum I was able to examine numerous West Coast collections of eunicids. Collections which had accumulated in the University of California over many years also yielded valuable material.

Six species of *Eunice* s. str. are known from California. They are

1. *E. biannulata* Moore, 1904. . . . widely known throughout the northeast Pacific, from Alaska south to San Diego, California.

Includes *Leodice valens* Chamberlin, 1918, 1919b. Chamberlin's type was collected near Mendocino, California, well within the range of *E. biannulata* Moore. Typical parapodia from the holotype of *Leodice valens* (figs. 8, 9) are strikingly like those of *E. biannulata* Moore. The appendages of the composite setae (fig. 22) are unique in this species. The descriptions by Moore (1904) and Chamberlin (1918, 1919b) disclose no material differ-



Figs. 12-15. *Eunice longicirrata* var.

Fig. 12. Carriers and forceps of maxillae from dorsal side. $\times 18$.

Fig. 13. Fifteenth parapodium. Setae omitted. $\times 8$.

Fig. 14. Composite hooded seta from a posterior parapodium. $\times 315$.

Fig. 15. Acicular seta from the same parapodium. $\times 315$.

Fig. 16. *Eunice hawaiiensis*. Acicular seta from a posterior parapodium. $\times 315$.

Figs. 17-21. *Eunice enteles*. All setae taken from posterior parapodia. $\times 315$.

Fig. 17. Acicular seta from individual from southern California. Accessory teeth large, on a short basal extension.

Fig. 18. Same, from another individual. Accessory teeth on a long basal extension.

Fig. 19. Acicular seta from individual from northern California. Accessory teeth on a long basal extension.

Fig. 20. Composite hooded setae, from the same parapodium from which fig. 18 was made.

Fig. 21. Composite hooded seta, from the same parapodium from which fig. 17 was made.

Figs. 22-23. *Eunice biannulata*. Both setae from the same posterior parapodium. $\times 315$.

Fig. 22. Composite hooded seta.

Fig. 23. Acicular seta.

ences. The largest individuals I have seen were collected by Mr. E. F. Ricketts in Alaska, near Sitka. These are 20 cm. long, preserved.

2. *E. enteles* (Chamberlin), 1918. northern and southern California.

Includes *Leodice monilifer* Chamberlin (1919a). The only differences in the descriptions of *E. enteles* and *L. monilifer* concern the shapes of the tentacular cirri and the disposition of the branchiae. Both of these characters are variable to a high degree. The type locality of *E. enteles* is Mendocino; that of *L. monilifer*, Laguna Beach. I have examined specimens from Laguna Beach and from Moss Beach (San Mateo County). These are identical. Acicular setae and composite hooded setae of some of these individuals are shown in figures 17-21.

3. *E. hawaiiensis* Treadwell, 1906. southern California; Hawaii.

4. *E. kobiensis* McIntosh, 1885. north Pacific, east and west.

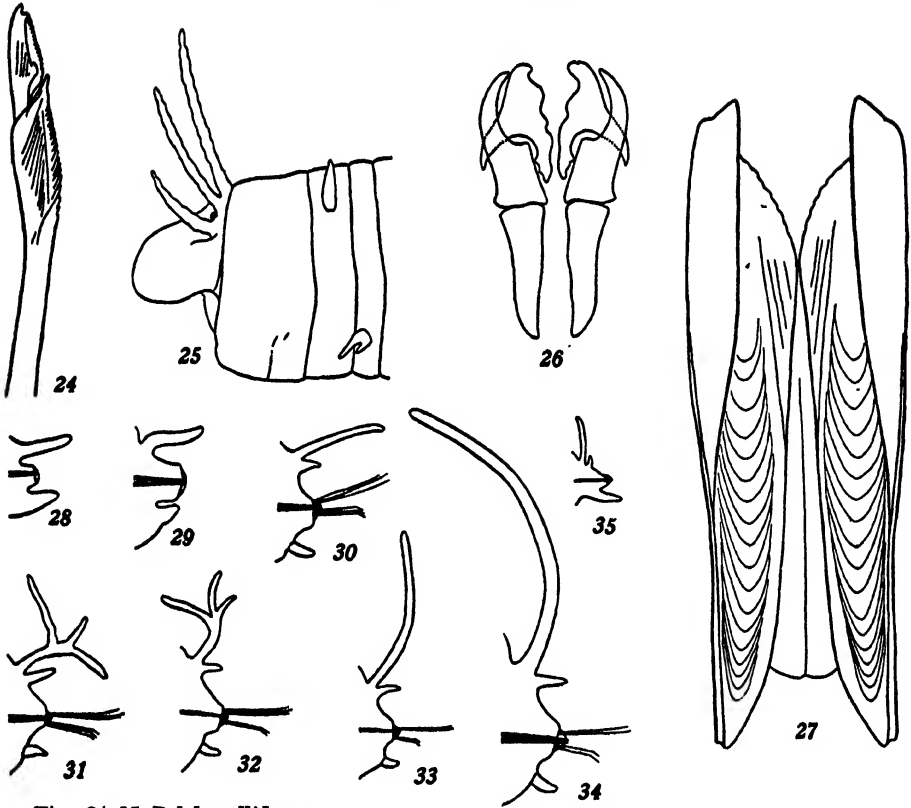
5. *E. longicirrata* Webster, var. (see below) central California.

Berkeley (1935) has already reported this species from Elkhorn Slough. I have examined several individuals, collected from Pacific Grove, which compare favorably with the description of this species as given by Treadwell (1921), but which differ in a few details. The dorsal cirri are not as long as the branchial filaments in the branchial region (fig. 13). The unpaired maxillary piece has 7 teeth, not 6, and the distal paired pieces (iv) have 7 and 10, respectively, instead of 8 and 10. The carriers of the forceps are more rectangular at their proximal ends (fig. 12). The median and inner lateral prostomial antennae have 14 and 15 articles, respectively; the outer lateral tentacles have about 7 articles. The tentacular cirri have 5 to 7 irregular articulations. The specimens are characterized by a pale band across the third podous segment. Setae are as shown in figures 14 and 15. Because of these differences, I have designated the individuals from Pacific Grove a variety of the North Atlantic species.

6. *E. multipectinata* Moore, 1911. central California (dredged).

Key to the Species of *Eunice* from the Northeast Pacific

1. Acicula and acicular setae (figs. 15, 16) pale or yellowish; branchial filaments not bifid; acicular setae bi- or tridentate. 2
1. Acicula and acicular setae dark brown; branchial filaments bifid; acicular setae bidentate. *E. multipectinata*
2. Acicular setae tridentate (figs. 16, 17) 3
2. Acicular setae bidentate (figs. 15, 23) 4
3. Distal teeth of acicular setae without basal extension (fig. 16); branchial region short, though clearly marked because of the development of the branchiae, present from segments 2 or 3 and attaining about 30 or more pinnae where best developed
E. hawaiiensis
3. The two distal teeth of the acicular setae with a basal extension (figs. 17-19); branchial filaments few, not exceeding 4 or 5 where best developed (figs. 10, 11)
E. enteles
4. Prostomial antennae strongly moniliform, the median and inner lateral antennae consisting of 14 or 15 articles; dorsal cirri less distinctly articulated (fig. 13); pale band across third podous segment. *E. longicirrata*
4. Prostomial antennae articulated more or less strongly but not moniliform; dorsal cirri smooth; without pale band across third podous segment. 5
5. Distal teeth of composite hooded setae poorly developed (fig. 22); branchiae first present from segments 6 or 7; articulations of prostomium continued through basal half *E. biannulata*
5. Distal teeth of composite setae normally developed; branchiae first present on segment 3; articulation of prostomial antennae limited to distal half. *E. kobiensis*



Figs. 24-35. *Palolo pallidus*.

Fig. 24. Falcigerous seta from a median parapodium. $\times 340$.

Fig. 25. Anterior end from left side. Mandible partly protruded. $\times 9$.

Fig. 26. Maxillae: Carriers and I (forceps) and II in dorsal view. $\times 21$.

Fig. 27. Mandibles in dorsal view. $\times 21$.

Figs. 28-35. Parapodia in anterior view. Setae diagrammatic where shown. All $\times 21$.

Fig. 28. Second setigerous segment.

Fig. 29. Tenth setigerous segment.

Fig. 30. First branchial (117th setigerous) segment.

Fig. 31. Tenth branchial segment.

Fig. 32. Fourteenth branchial segment.

Fig. 33. Twenty-fifth branchial segment.

Fig. 34. A parapodium from middle of branchial region.

Fig. 35. Last branchial or sixth before last setigerous segment.

Genus *Palolo* Gray, 1847

Diagnosis.—Differs from *Eunice* in that the branchiae are present only in a posterior region; branchial filaments simple, at most consisting of 1 to a few branches; without the acicular setae and pectinated setae characteristic of *Eunice*; jaw pieces consisting of a heavy, stout mandible greatly exceeding the maxilla in size (figs. 26, 27); teeth of maxillae poorly developed (fig. 26). Swarming and epitoky known for some species.

Type.—*Palolo viridis* Gray, 1847. Includes also the widely known *Eunice sicilensis* Grube.

Type locality.—Samoa (Navigator Islands).

Key to Species of *Palolo* from California

- Branchiae simple, first present as minute papillae, others cirriform; acicula 3 or 4 in a parapodium in anterior region. *P. paloloides* (Moore)
- Branchiae in part branched (figs. 31, 32); first branchiae cirriform (fig. 30); acicula not more than 2 in a parapodium. *P. pallidus* sp. nov., p. 99

Palolo pallidus sp. nov.
(figs. 24-35)

Diagnosis.—Length 20-30 cm.; width 4-5 mm.; long, slender, readily autotomizing in life. Segments consist of 115-141 prebranchial, and 250-300 branchial, or a total of about 400-500 segments.

Prostomium $1\frac{1}{2}$ times as broad as long, its length exceeding that of the dorsal side of the peristomium; its anterior median emargination about $\frac{1}{4}$ as deep as prostomium is long; a shallow, median groove continued to the peristomial ring. Prostomial antennae with irregular, shallow wrinkles (fig. 25), the median antenna longer than the prostomium, the ventrolaterals as long as, or slightly shorter than, the prostomium. Eyes 2, dark brown, inserted between bases of paired dorsal and ventral antennae (fig. 25).

Peristomium about twice as long as segment 2, longest along its ventral side; lower lip smooth. Second segment about $1\frac{1}{2}$ times as long as segment 3, its cirri smooth, their length slightly exceeding that of the segment (fig. 25).

Parapodia ventrolateral in position. First parapodium with dorsal and ventral cirri larger than those of next few segments, its setae and acicula emerging from the crotch formed by the cirri. Second parapodium with the setigerous lobe well developed, the two black acicula emerging from a fleshy lobe dorsal to the falcigerous setae and anterior to the capillary setae. Dorsal cirrus exceeding ventral cirrus in length, but the latter thicker. Posterior to the 4th parapodium, the ventral side of the neuropodium gradually acquires a thickened glandular pouch, these pouches continuing large through the prebranchial region and through about 60 branchial segments.

A typical prebranchial parapodium with 2 black acicula, 4-6 superior capillary setae, and 8-12 falcigers (fig. 24). Disposition of setae in branchial segments similar, but setae present in diminishing numbers. Most branchial segments with a single aciculum, a few with 2 acicula (figs. 31, 34).

Branchiae first present from segments 115-141, continued posteriorly almost to the posterior end, absent from last 6 or 7 segments; chiefly simple, cirriform, a few irregularly branched (figs. 31, 32), the branches not exceeding 3 or 4 pinnae where best developed.

Mandibles with heavy, calcareous, deeply ridged, angular paired pieces which are held together along their inner margins by a chitinized membrane (fig. 27); the anterolateral margins curved upward and inward and terminating distally in a smooth heavy tooth (fig. 27); ventral free margin crenulate.

Maxillae deep brown or dusky, the carriers almost as long as the forceps (fig. 26), main pieces as in figure 26. Pygidium a simple ring; ventral anal cirri slightly longer than the pygidium is broad.

Color in life pale salmon, deepest anteriorly, paler in setigerous region.

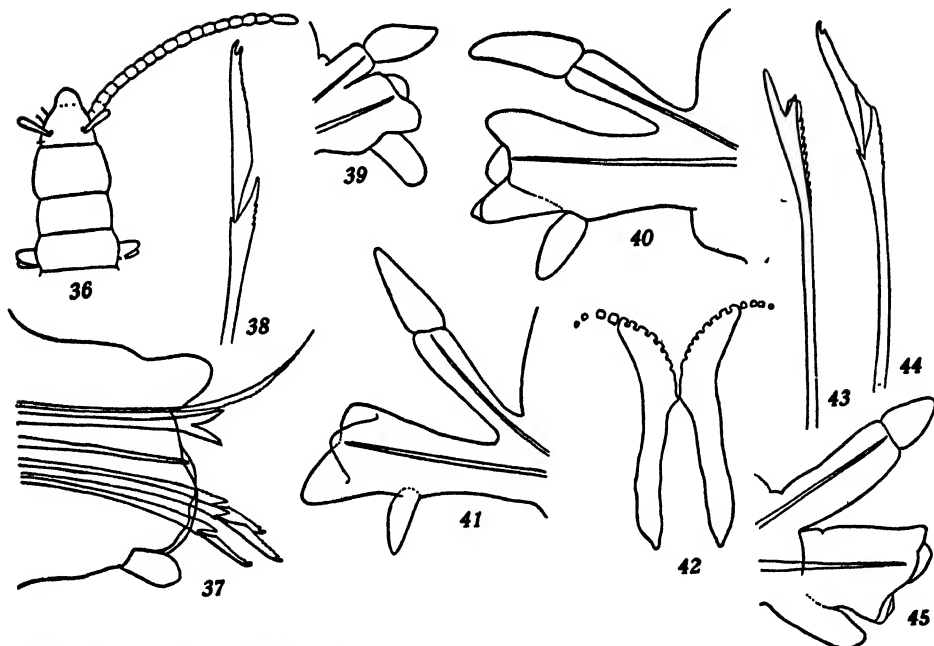
Holotype.—U. S. Nat. Mus. no. 20362.

Distribution.—Laguna Beach (type), California. Common in vermetid colonies and kelp holdfasts in low-tide region.

Discussion.—*P. pallidus* approaches *P. leucodon* (Ehlers), from Juan Fernandez, off the coast of Chile. The latter is generally considered a synonym of *P. siciliensis* (Grube) (*vide* Fauvel, 1911, p. 406). The jaw pieces bear similarities; the branchiae, however, are different in that some are branched; also, anterior parapodia are biacicular. *P. pallidus* differs from *P. paloloides* (Moore), as indicated in the foregoing key.

Family STAUONEREIDAE

A single genus is known to be represented in California. Only one species, *Stauronereis moniloceras* Moore, has been described. Two other species are herein added, *S. gracilis* sp. nov. and *S. articulatus* sp. nov.



Figs. 36-38. *Stauronereis gracilis*

Fig. 36. Anterior end including first 3 segments in dorsal view. Left anterior antenna not shown. $\times 54$.

Fig. 37. A median parapodium, setae indicated. $\times 54$.

Fig. 38. Distal end of a composite seta. $\times 630$.

Figs. 39-44. *Stauronereis articulatus*

Fig. 39. Second parapodium in anterior view. $\times 90$.

Fig. 40. A median parapodium in posterior view. $\times 90$.

Fig. 41. A posterior parapodium in anterior view. $\times 90$.

Fig. 42. Mandibles, including accessory pieces. $\times 90$.

Fig. 43. A superior neuroseta from a median parapodium. $\times 360$.

Fig. 44. An inferior neuroseta from a median parapodium. $\times 360$.

Fig. 45. *Stauronereis moniloceras*. A median parapodium. $\times 22$.

Genus *Stauronereis* Verrill

Includes *Staurocephalus* Grube, 1855, *non* Barrande 1846 (Crustacea); *Anisoceras* Grube 1856, *non* Dejean 1833 (Coleoptera); *Prionognathus* Keferstein 1862, *non* Laferté 1851 (Coleoptera); *Dorvillea* Parfitt 1866, *non* Leach 1815 (Lepidoptera).

Key to Species of *Stauronereis* from California

1. Notopodia with acicula and cirrophores (fig. 40) 2
1. Notopodia reduced, without acicula or cirrophores (fig. 37) *S. gracilis*, p. 100
2. Larger, brilliantly banded with coral and cream stripes; prostomial antennae thick, blunt; median neuropodia longest in their dorsal regions (fig. 45) . *S. moniloceras* Moore
2. Small, pale or colorless; prostomial antennae long, slender, articulated; median neuropodia longest in their ventral regions (fig. 40) *S. articulatus*, p. 101

Stauronereis gracilis sp. nov.

(figs. 36-38)

Diagnosis.—Length 8-15 mm.; width 0.5-0.8 mm.; number of segments 50-60. Minute, slender; convex dorsally, flattened ventrally; the segments about $\frac{2}{3}$ as long as wide and distinctly annulated throughout.

Prostomium conical, tapering anteriorly and terminating in a small semicircular flap which is slightly set off by a faint transverse line from the rest of the prostomium (fig. 36); one pair of long anterior antennae with 10–18 shallow annulations (fig. 36), terminal annulus the longest. A pair of short, simple antennae more posteriorly, with eyespots at their bases (fig. 36).

Segments 1 and 2 achaetous and apodous, the first segment larger and somewhat inflated (fig. 36). Parapodial segments similar throughout, diminishing in size gradually in posterior segments. Parapodia conspicuous, projecting laterally or somewhat posterolaterally; basal stalk about $\frac{1}{2}$ as long as body width, truncate distally; pre- and post-setal lobes both short, about equal in length (fig. 37). With three kinds of setae (fig. 37). A typical parapodium contains a pointed seta, a single, simple forked dorsal seta, 2 or 3 composite falcigerous setae ventrally, and a single aciculum. Composite setae with a bidentate appendage and a toothed shaft (fig. 38). Dorsal and ventral cirri simple, conical, the dorsal cirri lacking cirrophores and aciculum.

Anal cirri 4, simple, the two dorsal ones slightly longer than the anal segment, the two ventral cirri slightly shorter.

Color in life white or colorless.

Holotype.—U. S. Nat. Mus. no. 20364.

Distribution.—Moss Beach, San Mateo County (type); Caspar, Mendocino County; San Pedro, Los Angeles County. Common in sands at low-tide line.

Discussion.—*S. gracilis* approaches *S. kefersteini* McIntosh in its small size, in its prostomial proportions, and in the absence of notocirrophores and dorsal aciculum. It differs in having short, truncate setal lobes, in its simple posterior antennae, and in the anterior position of the eyes with respect to the antennae.

Stauronereis articulatus sp. nov.

(figs. 39–44)

Diagnosis.—Length 14–18 mm.; width up to 1.6 mm. with, 0.6 mm. without, parapodia. Number of segments about 50. Dorsum strongly convex; ventrum flat. Segments crowded, but well marked by intersegmental furrows. Prostomium subspatulate, broadly rounded anteriorly, slightly longer than broad; an anterior portion set off from posterior half by a faint white transverse line. Eyes 4, an anterior, larger pair just in front of the annulated antennae, a smaller pair situated just within, and slightly posterior to, the antennae. Palpi biarticulated, with a thick basal portion which is as long as the prostomium is wide, and a slender distal appendage less than half as long. Antennae distinctly moniliform, with about 7 articles; antennae extend distally somewhat beyond the palpi.

Maxillae consisting of about 50 pairs of horny, deep brown pieces, the lateral pieces smaller and paler. Mandibles deep brown, basal portion almost straight, elongate (fig. 42), the free edge with 7–9 teeth and 3–5 detached pieces.

First two segments apodous and achaetous, slightly longer than segments which follow; parapodia relatively long throughout, longest in region just anterior to middle of body; dorsal cirri present on all parapodia but the first. Cirrophores long, slender, length exceeding twice width (figs. 39–41), each with slender aciculum. Cirrostyles more than half as long as cirrophores. Neuropodia conspicuous, superior and inferior anterior lobes diverging distally (fig. 40). Anterior neuropodium with a single stout aciculum and three kinds of setae: (1) 3–5 simple forked setae, (2) pointed setae, and (3) 6–8 composite setae in the inferior portions of the fascicles. Posterior neuropodia without the pointed setae. Composite setae with an appendage bidentate at the tip and toothed along one margin; shaft with 3 to 5 teeth near the outer distal margin (fig. 44).

Holotype.—U. S. Nat. Mus. no. 20363.

Distribution.—Dillon Beach, Marin County (type); Point Conception, Santa Barbara County, south to San Pedro, Los Angeles County.

Discussion.—Conspicuous features of *S. articulatus* are (1) the greatly elongated parapodial lobes, (2) the moniliform antennae, and (3) the nature of the parapodial structures. *S. articulatus* differs from *S. rudolphi* (delle Chiaje), which it approaches in some respects, (1) in the form of the neuropodial lobes, which are broadest distally in *S. articulatus*, and (2) in the structure of the second parapodia, in which the inferior portion of the neuropodium exceeds the superior portion in length.

Family OPHELIIDAE

Nine species in six genera of Opheliidae have heretofore been reported from the northeast Pacific. Of these, one (*Ammotrypane gracile*, Treadwell) is probably a synonym of another (*A. aulogaster* Rathke); two (*Ophelina magna* and *O. mucronata* Treadwell) belong to the genera *Ophelia* Savigny and *Thoracophelia* Ehlers, respectively; another (*Polyophthalmus australis*, Treadwell) is probably the cosmopolitan *P. pictus* (Dujardin). With these emendations, seven species in seven genera have been reported heretofore.

Extensive collections from many parts of California have brought to light two species not previously reported, and four species new to science, including a new genus. There are thus thirteen species in eight genera. Two of the new species have affinities with species recently described from northern Japan (Okuda, 1934, 1936) for which a new generic name (*Pectinophelia*) is herein proposed (p. 107). I wish to express appreciation especially to Professor O. L. Williams, of the College of the Pacific, and to Dr. Waldo L. Schmitt, of the United States National Museum, for making available numerous valuable collections; also to Dr. Shiro Okuda, of the Hokkaido Imperial University, for Japanese collections, including paratypes.

The following list includes the known species from the northeast Pacific:

1. *Ammotrypane aulogaster* Rathke, 1843. cosmopolitan; Alaska south to southern California.
Possibly includes *A. gracile*, Treadwell 1914, from southern California. *Non* McIntosh, 1885.
2. *Armandia brevis* (Moore) Alaska; western Canada; California.
3. *Ophelia magna* (Treadwell) southern California.
Ophelina magna Treadwell, 1914 (see p. 107).
4. *Thoracophelia mucronata* (Treadwell) northeast Pacific.
Ophelina mucronata Treadwell, 1914; Berkeley, 1932. This species has the characters of the genus *Thoracophelia* as defined by Ehlers, 1897.
5. *Dindymenides granulata* (Moore) northeast Pacific.
Travisia granulata Moore, 1923; Berkeley, 1929. Moore (1923) described this species as a *Travisia* but recognized it as a species of *Dindymene* Kinberg. The latter was changed to *Dindymenides* Chamberlin, *Dindymene* being preoccupied in Crustacea.
6. *Travisia brevis* Moore, 1906. northeast Pacific.
7. † *Polyophthalmus australis*, Treadwell, 1914. southern California.
See note under *P. pictus* (below).
The following are new records for the northeast Pacific and new species:

8. *Polyopthalmus pictus* (Dujardin) . . . cosmopolitan; southern California.
P. australis, Treadwell, from southern California, may be this species.
9. *Armandia bioculata* sp. nov. (see p. 105) northern California.
10. *Ophelia limacina* (Rathke) (see p. 107) North Pacific, east and west.
11. *Pectinophelia dillonensis* sp. nov. (see p. 108) . Marin County, California.
12. *Pectinophelia williamsi* sp. nov. (see p. 109) . . . Marin County, California.
13. *Travisia gigas* sp. nov. (see p. 103) northern and southern California.

Key to the Genera of OPHELIIDAE from the Northeast Pacific

1. Ventral groove present, well marked. 3
1. Ventral groove absent. 2
2. A pair of setal fascicles anterior to mouth; segments triannulate, the rings about equally long; epithelium more or less smooth or papillated. *Travisia*, p. 103
2. Without setal fascicles anterior to mouth; segments triannulate but with middle or setigerous ring distinctly the larger; epithelium crowded with surface pustules
Dindymenides (*D. granulata*)
3. Ventral longitudinal groove extending throughout length. 4
3. Ventral longitudinal groove in posterior region only. 6
4. Cirriform branchiae present on some parapodia. 5
4. Parapodia without branchiae. *Polyopthalmus* (*P. pictus*)
5. Lateral eyespots between some successive parapodia. *Armandia*, p. 105
5. Without lateral eyespots. *Ammotrypane* (*A. aulogaster*)
6. Anterior region including first 2 setigerous segments, set off from thorax by a median constricted ring (fig. 60); anal cirri consisting of a larger median ventral cirrus and numerous dorsolateral cirri disposed in an inverted V; 5 pairs of nephridial pores, situated about midway between parapodial ridges. 7
6. Anterior region not set off from thoracic region by a constriction (fig. 55); anal cirri consisting of a pair of larger ventral papillae and several smaller dorsal papillae disposed in a transverse series (figs. 53, 56); 6 pairs of nephridial pores, situated on or near the parapodial ridges. *Ophelia*, p. 106
7. Branchial cirri forked, consisting of 2 simple, cirriform branches. . *Thoracophelia*, p. 107
7. Branchial cirri pectinately or compoundly branched (figs. 61-63) . . *Pectinophelia*, p. 107

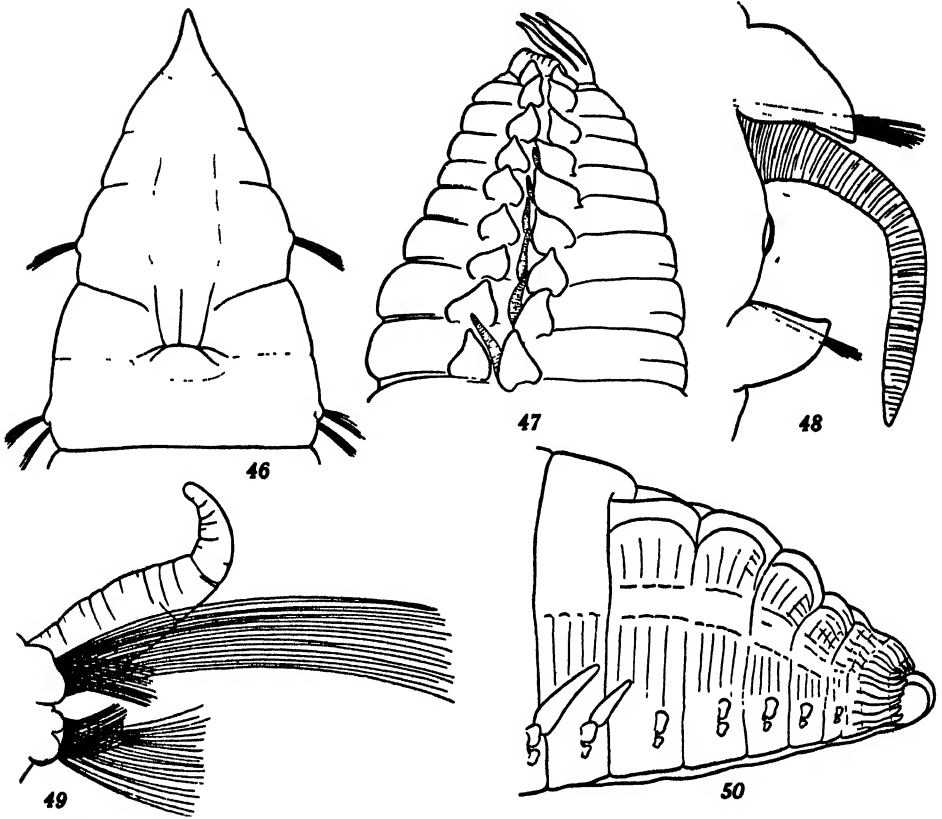
Genus *Travisia* JohnstonKey to the Species of *Travisia* from the Northeast Pacific

1. Parapodial lappets (fig. 47) large through the posterior region; consisting of 46 setigerous segments. *T. gigas*, p. 103
1. Parapodial lappets not present near the posterior end; consisting of fewer than 40 setigerous segments 2
2. Consisting of 29 setigerous segments; parapodial lappets on segments 15-23; anterior segments without large vesicles. *T. brevis* Moore
2. Consisting of 31-32 setigerous segments; without parapodial lappets; anterior and posterior segments with short, transverse series of large vesicles dorsal and ventral to the parapodia. *T. pupa* Moore

Travisia gigas sp. nov.

(figs. 46-48)

Diagnosis.—Length 50-100 mm.; width up to 13 mm.; thickest between setigerous segments 7 and 14; elongate, grublike; number of setigerous segments 46. Epithelium smooth except for minute papillations which are visible under magnification. Segments triannulate,

Figs. 46-48. *Travisia gigas*Fig. 46. Anterior end in ventral view. $\times 7.5$.Fig. 47. Posterior end from right side. $\times 7.5$.Fig. 48. Seventeenth setigerous segment in anterior view. $\times 37$.Figs. 49-50. *Ophelia magna*Fig. 49. Fifteenth parapodium in anterior view. $\times 18$.Fig. 50. Seven last setigerous segments and pygidium, from left side (setae omitted). $\times 7.5$.

the rings equal or subequal dorsally and ventrally, but irregular in region of parapodia. Prostomium conical, longer than broad, nuchal organs almond-shaped, situated at dorso-lateral, posterior margin of prostomium.

Branchiae present from second setigerous segment, continued posteriorly to third from last segment; simple, cirriform, very contractile; largest between segments 15-25, the last few tiny, concealed between the parapodial lappets (fig. 47). Twelve pairs of subparapodial sense organs situated on setigerous segments 3-14. Prominent triangular parapodial lappets (fig. 48) beginning in segment 15, inserted dorsal and ventral to the setal fascicles and continued with slight and gradual increase in size to about the 35th setigerous segment, decreasing in size more posteriorly, but remaining conspicuous to the posterior end. Like the parapodia, the lappets come to approach one another (fig. 47) so that in the last 5 or more segments they are almost adnate along their medial margins.

Second setigerous segment faintly biannulate; next 2 segments indistinctly triannulate; segments 5-15 more or less completely triannulate. The 3d annulus begins to fade out from the 16th segment and remains only as a dorsal and a ventral mark to about the 25th segment. After that, segments are biannulate and become strictly uniannulate in the last 5 segments.

Setae soft, capillary, numerous (up to 20 in a fascicle) but not prominent. Pygidium a

broad collar, about as broad as last 2 segments, the ventral half marked with longitudinal wrinkles, the dorsal half with 6 long cirri (fig. 47), which may, however, be contracted and short. Within the collar are numerous small conical papillae.

Holotype.—U. S. Nat. Mus. no. 20365.

Distribution.—San Diego, California (type); Tomales and San Francisco bays.

Discussion.—*Travisia gigas* compares with *T. elongata* Grube in the large number of its segments (46) and in having conspicuous parapodial lappets. According to Ehlers (1901, p. 171), and contrary to Grube (1866, p. 66), *T. elongata* comes from northern Chile (Iquique), not Samoa as stated by Grube. The two species are readily distinguished in the following respects: (1) *T. gigas* has branchiae from the 2d segment to the next to last segment or 44 pairs, *T. elongata* has only 28 pairs; (2) parapodial lappets of *T. gigas* are first present in noto- and neuropodia in the 15th segment, in *T. elongata* from the 20th notopodium and 26th neuropodium posteriorly; (3) the pygidium of *T. gigas* is provided with 6 long cirri, whereas no cirri have been described for *T. elongata*; and (4) *T. gigas* may be 100 mm. long, *T. elongata* only 29 mm. long.

Genus *Armandia* Filippi

Key to the Species of *Armandia* from the Northeast Pacific

- Lateral eyespots crescentic; lateral lobes of anus obliquely truncated above and slightly indented at the end.....*A. brevis* (Moore)
 Lateral eyespots circular; lateral lobes of anus terminating in 5 dorsal and 2 sublateral papillae (figs. 52, 53).....*A. bioculata* p. 105

Armandia bioculata sp. nov.

(figs. 51–54)

Diagnosis.—Length up to 17 mm.; width up to 1.2 mm.; number of setigerous segments 29; slender, terete, tapering gradually toward both ends. Surface smooth except for annulations. Prostomium depressed conical, longer than wide, with a slender anterior portion projecting forward or ventrally (fig. 51). Nuchal organs immediately anterior, and somewhat dorsal, to first setigerous segment (fig. 51). A pair of black, deep-seated eyespots, anteroventral to the nuchal organs.

Proboscis (everted) elongate, sacklike (fig. 51); mouth crescent-shaped when proboscis is retracted, upper lip with a median notch, lower lip with 4 longitudinal furrows.

Parapodia with well-developed presetal lamellae and setal fascicles (fig. 54). Setae silky, those of first 4 or 5 and the last parapodia exceeding others in length. Tenth parapodium with 8–10 notosetae and 5–7 neurosetae, the neurosetae only half as long as the notosetae. Presetal lamella consisting of a blunt dorsal lobe and a slenderer prolongation along its ventral margin (fig. 54); ventral cirrus shorter, bluntly rounded, somewhat posterior and ventral to parapodial lamellae (fig. 54).

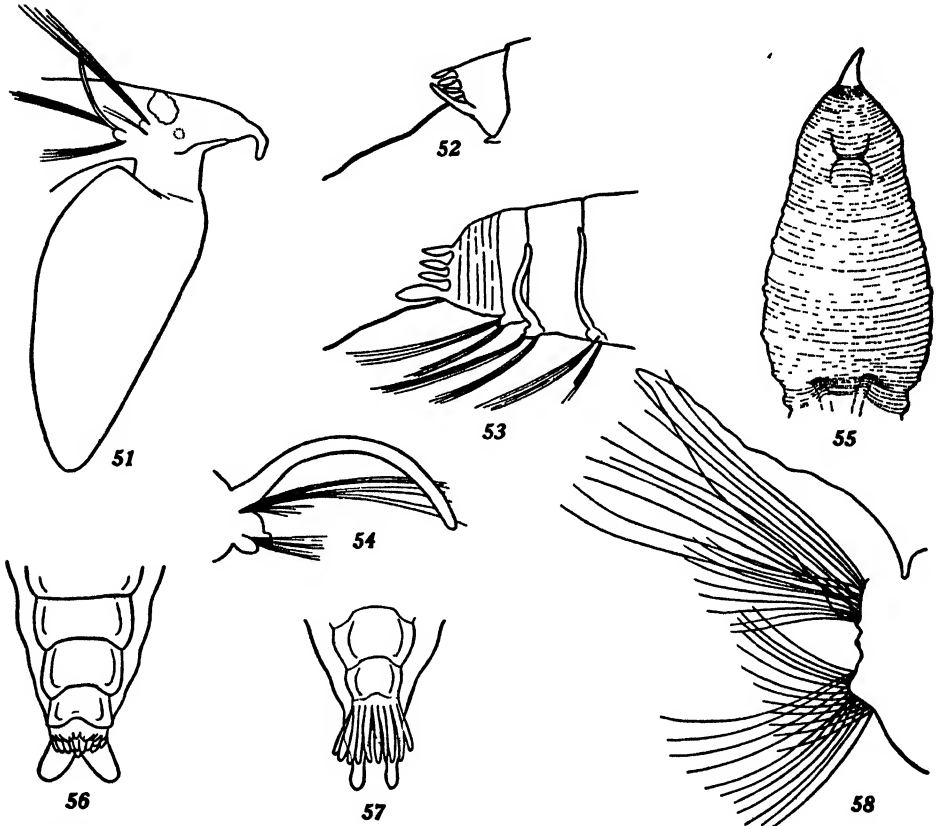
Branchiae 27 pairs, present from 2d setigerous segment to the next to the last, or 28th, segment; branchiae long, smooth, cirriform, extending distally beyond the setae except in the first and last five setigerous segments, where the setae are longer than elsewhere and exceed the branchiae in length. Pygidium an achaetous ring (figs. 52, 53), terminating in a transverse row of 5 dorsal and 2 ventrolateral cirri, the latter more than twice the size of the former. Anus with a threadlike, slender filament, characteristic of the genus. Lateral eyespots circular, present between setigerous segments $\frac{9}{4}$ to $\frac{19}{4}$, a total of 11 pairs.

Includes numerous individuals agreeing in all characters with the foregoing description except for their shorter, blunter appearance (up to 15 mm. long). Anal funnel more triangular (fig. 52) but with a similar arrangement and distribution of anal cirri.

Holotype.—U. S. Nat. Mus. no. 20366.

Distribution.—Moss Beach, San Mateo County (type); Drake's Estero; northern California.

Discussion.—*A. bioculata* differs from *A. brevis* (Moore) in the following respects: (1) The lateral eyespots are circular; (2) branchiae are absent from the last 2 parapodia; (3) pygidial cirri consist of 5 slender, dorsal, and 2 larger ventrolateral cirriform cirri; and (4) the prostomium has 2 eyes.



Figs. 51-54. *Armandia bioculata*

Fig. 51. Anterior end from the right side, with proboscis and nuchal organs everted. $\times 20$.

Fig. 52. Posterior end from a short individual, from right side. $\times 20$.

Fig. 53. Pygidium from a long individual, from right side. $\times 20$.

Fig. 54. Ninth setigerous segment in posterior view. $\times 40$.

Figs. 55-58. *Ophelia limacina*

Fig. 55. Anterior thoracic region in ventral view. Setae omitted. $\times 8$.

Fig. 56. Posterior end in dorsal view; individual with greatly contracted anal cirri. $\times 8$.

Fig. 57. Same, from an individual with elongate cirri. $\times 8$.

Fig. 58. Seventh abdominal segment in anterior view. $\times 35$.

Genus *Ophelia* Savigny

Key to the Species of *Ophelia* from the Northwest Pacific

- Larger, more than 80 mm. long; with 31 pairs of branchiae; pygidium with 2 spherical, ventral papillae and about 10 pairs of tiny dorsal papillae (fig. 50); 2d and 3d abdominal segments with porous transverse ridges. *O. magna*, p. 107
- Smaller, length not exceeding 75 mm.; with less than 25 pairs of branchiae; ventral anal papillae elongate (figs. 56, 57); 2d and 3d abdominal segments without ridges. *O. limacina*, p. 107

Ophelia magna (Treadwell)*Ophelia magna* Treadwell, 1914

(figs. 49, 50)

Additional description.—Parapodial lobes with broad, fleshy presetal lobes; postsetal neuropodial lobe bifid, pierced by a pore (fig. 49). Postbranchial region consisting of 5 setigerous segments and the pygidium; surface thrown into two strong dorsolateral longitudinal ridges (fig. 50) in addition to the ventral groove, that terminate dorsally in a semicircular series of short papillae and ventrally in two stout spherical papillae (fig. 50).

Known only from southern California.

Ophelia limacina (Rathke)

(figs. 55–58)

Numerous individuals from northern and southern California agree with descriptions of the North Atlantic *O. limacina*. These specimens have 7 thoracic setigerous segments, 2 prebranchial segments, 17–24 branchial segments, 4–5 postbranchial segments, and the pygidium. The pygidial cirri are short, blunt (fig. 56), to long, cirriform (fig. 57), depending on the degree of contraction of the specimen. Two individuals from Sapporo, Japan (courtesy of Dr. Shiro Okuda) belong to this species.

Genus *Thoracophelia* Ehlers

Discussion.—In 1897, Ehlers described a member of the family Opheliidae from the Straits of Magellan, for which he erected the genus *Thoracophelia*. In 1914, Treadwell described from California a species belonging to the same genus but placed it in the genus *Ophelia* (Ersed). The latter is a synonym of *Ammotrypane* Rathke, hence not available for this group (*vide* Fauvel 1927, p. 133).

More recently, Okuda (1934, 1936) described two species of *Thoracophelia* (*Th. esoensis* and *Th. yasudai*) from northern Japan. Both of these species differ from Ehlers' *Thoracophelia* in several fundamental respects, but have notable similarities with two new species of *Pectinophelia* gen. nov. described below. There are, therefore, only two known species belonging to *Thoracophelia* Ehlers. These are separable as follows:

Twenty pairs of branchiae and 6 posterior abbranchiate segments; anus with 5 pairs of dorsal papillae.....*Th. furcifera* Ehlers
Eighteen pairs of branchiae and 8 posterior abbranchiate segments; anus with 7 pairs of dorsal papillae.....*Th. mucronata* (Treadwell)

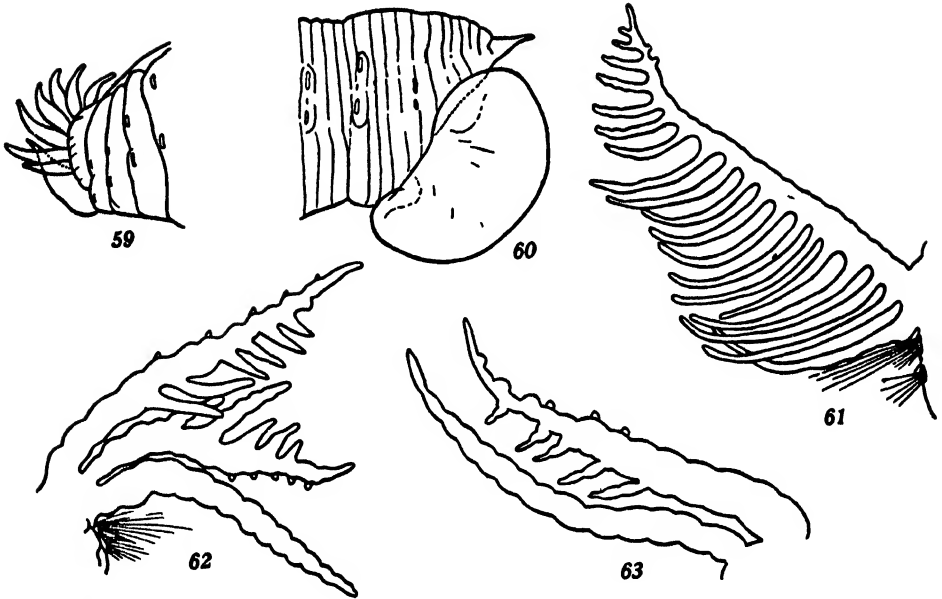
Genus *Pectinophelia* nov.

Type of genus.—*P. dillonensis* sp. nov.

Branchial cirri compound, with lateral pinnae; parapodia greatly reduced; 5 pairs of nephridial papillae, disposed between successive neuropodial ridges on the 12th to 16th setigerous segments. Differs from *Thoracophelia* Ehlers in having compoundly divided branchial cirri, and in having the parapodia less developed.

Key to the Species of *Pectinophelia* from the North Pacific

1. Branchial cirri dendritically branched.....*P. yasudai* (Okuda)
1. Branchial cirri pectinately divided..... 2
2. Branchial pinnae short, irregular, arising from both sides of rachis (figs. 62, 63)....
P. williamsi, p. 22
2. Branchial pinnae neatly pectinate, long, slender (fig. 61)..... 3
3. With 19 pairs of branchiae; 5 pairs of lateral anal cirri; branchiae with about 13 pinnae at their greatest development.....*P. esoensis* (Okuda)
3. With 15 pairs of branchiae; 7 pairs of lateral anal cirri (fig. 59); branchiae with about 20 pinnae where best developed.....*P. dillonensis*, p. 108



Figs. 59-61. *Pectinophelia dillonensis*

Fig. 59. Posterior end from right side. Setae omitted. $\times 9.7$.

Fig. 60. Anterior end with proboscis everted, from same individual. Setae omitted. $\times 9.7$.

Fig. 61. Tenth branchial segment in posterior view. (Most of setae omitted.)

Figs. 62, 63. *Pectinophelia williamsi*

Fig. 62. Eighth branchial parapodium. $\times 25$.

Fig. 63. Branchia from midbranchial parapodium. $\times 25$.

Pectinophelia dillonensis sp. nov.

(figs. 59-61)

Diagnosis.—Length up to 70 mm.; width up to 3.5 mm.; slender, subcylindrical except for ventral groove. Distribution of segments as follows: head with 2 setigerous segments, thorax with 8 segments, abdomen with 2 abbranchiate, 15 branchial, and 11 postbranchial segments, total of 38 setigerous segments. Surface glabrous except for weak annulations.

Prostomium a minute pointed cone (fig. 60) about 3 times as long as broad at its base. Eight poorly marked annuli precede the dorsolateral nuchal slits. Transverse mouth slit bounded by broad, smooth upper and lower lips.

Parapodia biramous, greatly reduced in size, parapodial lamellae tiny in neuropodia (fig. 61), not discernible in notopodia. Simple capillary setae and 3 or 4 reduced acicula in each podium. Notoetae and neuroetae similarly arranged, each ramus with an anterior series of 10-12 shorter, finer setae, and a posterior series of 8-11 stouter, longer setae. Five pairs of nephridial pores, on setigerous segments 12-16, situated slightly posterior to middle of segment, between successive neuropodial ridges. Interramal pores minute, crescent-shaped. Glandular ridge on last thoracic (10th setigerous) segment elongate, only slightly elevated, smooth, without visible pores.

Postbranchial segments consisting of 7 segments which are longer than broad, and 4 short telescoped segments which narrow rapidly to the anal segment (fig. 59). Setal ridges of last 4 segments successively more ventral in position, their rami approaching one another (fig. 59). Setae of thoracic segment and of last 4 abdominal segments more conspicuous than those of other parapodia.

Branchiae pectinately branched, with 15-20 pinnae inserted along the posterior border of the rachis, and a few small lobes along the anterior distal margin (fig. 61).

Pygidium with 2 thick, tapering ventral cirri and 7 pairs of dorsal cirri disposed in an inverted V (fig. 59).

Holotype.—U. S. Nat. Mus. no. 20368.

Distribution.—Dillon Beach, Marin County, California (type). In low-tide zone, sandy beach.

Discussion.—*P. dillonensis* differs from its closely related species, *P. ezoensis* (Okuda), as indicated in the foregoing key (p. 108).

Pectinophelia williamsi sp. nov.

(figs. 62, 63)

Diagnosis.—Length up to 70 mm.; width up to 3.5 mm.; slender, subcylindrical except for ventral groove. Distribution of segments as follows: head with 2 setigerous segments, thorax with 8 segments, abdomen with 2 abbranchiate, 16 branchial, and 10 postbranchial segments, total of 38 setigerous segments.

Branchiae 16 pairs, consisting of 2 (rarely 3) equally long main branches, the ventral-most branch simple, cirriform or with margin somewhat crenulate (figs. 62, 63); the dorsal-most branch (or branches) irregularly pinnately divided, a series of longer pinnae inserted on the side proximal to the ventral cirriform branch, and a few much shorter pinnae inserted on the opposite side (figs. 62, 63). The 16th or last pair of branchiae smaller than the others and usually simply bifid or with only slight elevations on the dorsal branch. Strikingly like *P. dillonensis* (p. 20) in other respects.

This form is named for Professor O. L. Williams, of the College of the Pacific, Stockton, California, who collected numerous individuals of this and the preceding species from Bodega Bay, California.

Holotype.—U. S. Nat. Mus. no. 20367.

Distribution.—Dillon Beach, Marin County, California (type); in sandy beach with *P. dillonensis*.

Discussion.—*P. williamsi* is distinguishable from other closely related species as outlined in the foregoing key (p. 107).

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COMPARATIVE ECOLOGICAL STUDIES ON
THE TERRESTRIAL ISOPOD CRUSTACEA
OF THE SAN FRANCISCO BAY REGION

BY

MILTON A. MILLER

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INTRODUCTION

THE ONISCOIDEA, unlike the other five tribes in the order Isopoda, is one of the several groups of invertebrates which have migrated from the ocean and which have directly, through the intertidal zone, or indirectly, via fresh water, acquired terrestrial habitats. The terrestrial isopods present transitional stages in this migration from aquatic to land habitats. They are therefore not so advanced as are some of their relatives, notably the insects, in solving the problems of terrestrial existence, and are, in consequence, probably more affected by major environmental conditions. For these reasons, and also because they can easily be collected and maintained in the laboratory, they invite comparative ecological studies.

The author is indebted to Professor S. J. Holmes for suggesting this study, which was carried on under his supervision in the Department of Zoölogy at the University of California, and for his helpful criticism. Thanks are also due Professor E. A. Hoy, of the Department of Mathematics in the University of Hawaii, for valuable suggestions relative to the statistical analysis of the data.

PART I. NATURAL HISTORY

Twenty-seven species and three subspecies of terrestrial isopods, representing fourteen genera, have been reported from California. Of these, seventeen species and two subspecies are recorded from the San Francisco Bay region. In the following check list of California isopods, and in the subsequent artificial key to the species of the Bay region, asterisks indicate the species which we have collected in this region and the localities from which they were taken. In the reference column of the check list, no literature is cited prior to Richardson's classic monograph of 1905, which adequately summarizes previous work.

CHECK LIST OF THE ISOPODA OF CALIFORNIA

Species	Localities	References
* <i>Actoniscus lindahli</i> Richardson	*Alameda Oakland	Richardson (1905)
* <i>Actoniscus tuberculatus</i> Holmes and Gay	*Alameda San Diego	Holmes and Gay (1909)
<i>Alloniscus cornutus</i> Budde-Lund	California	Richardson (1905)
<i>Alloniscus cornutus lagunae</i> Stafford	Laguna Beach	Stafford (1913)
<i>Alloniscus mirabilis</i> (Stuxberg)	California	Richardson (1905)
* <i>Alloniscus perconvezus</i> Dana	*Moss Beach *Dillon Beach *Pescadero Pacific Grove Santa Barbara Monterey Bay Laguna Beach San Mateo	Richardson (1905) Stafford (1913) Arcangeli (1932)
<i>Armadillidium cinereum</i> (Zenk)	San Mateo	Arcangeli (1932)
* <i>Armadillidium vulgare</i> (Latreille)	*Berkeley *Oakland *Alameda *Moss Beach *(Widely distributed)	Richardson (1905)
<i>Armadillo (Diploezochus) microphthalmus</i> Arcangeli ¹	Saratoga	Arcangeli (1932)
<i>Cubaris affinis</i> (Dana) ¹	California	Richardson (1905)
<i>Cubaris californica</i> (Budde-Lund)	San Francisco San Pedro	Richardson (1905)
<i>Ligia exotica</i> Roux ²	California	Richardson (1905) Jackson (1922)
* <i>Ligia occidentalis</i> Dana	*Moss Beach *Montara *Tomales Bay *Laguna Beach *(Widely distributed along shores of San Francisco Bay, Monterey Bay, Farallones, Coast of California)	Richardson (1905) Jackson (1922) Stafford (1913)
* <i>Ligia pallasii</i> Brandt	*Montara *Fort Bragg Lagonistas Creek Farallones Cape Mendocino	Richardson (1905) Jackson (1922)
* <i>Ligidium gracilis</i> (Dana)	*Berkeley *Moss Beach *Eureka *Richardson's Grove	Richardson (1905) Jackson (1923)
<i>Ligidium hypnorum</i> (Cuvier) ³	California	Jackson (1923) Richardson (1905)
<i>Ligidium kofoidi</i> Maloney	Potter Creek Cave (Shasta County)	Maloney (1930)
<i>Ligidium latum</i> Jackson	San Francisco	Jackson (1923)
<i>Lyprobius pusillus</i> Budde-Lund	California	Richardson (1905)

CHECK LIST OF THE ISOPODA OF CALIFORNIA—(Continued)

Species	Localities	References
* <i>Metoponorthus pruinus</i> (Brandt)	*Berkeley *Oakland *Mount Diablo *San Diego	Richardson (1905)
* <i>Philoscia richardsonae</i> Holmes and Gay	*Alameda San Diego Laguna Beach	Holmes and Gay (1909) Stafford (1913)
<i>Porcellio formosus</i> Stuxberg ⁴	San Francisco San Pedro	Richardson (1905) Arcangeli (1932) Miller (1936)
* <i>Porcellio laevis</i> Latreille	*Berkeley *Oakland *Davis *Moss Beach Colfax Eldorado Monterey (Widely distributed)	Richardson (1905) Miller (1936)
* <i>Porcellio littorina</i> Miller	*Alameda	Miller (1936)
* <i>Porcellio scaber americanus</i> Arcangeli	*Carmel *Oakland San Mateo	Arcangeli (1932) Miller (1936)
* <i>Porcellio scaber scaber</i> Latreille	*Berkeley *Moss Beach San Francisco San Pedro Oakland Colfax Crescent City Lagonistas Creek	Richardson (1905) Arcangeli (1932) Miller (1936)
* <i>Porcellio spinicornis occidentalis</i> Miller	*Berkeley *Moss Beach	Miller (1936)
<i>Protrichoniscus heroldi</i> Arcangeli	Muir Woods	Arcangeli (1932)
<i>Tylos punctatus</i> Holmes and Gay	San Diego Laguna Beach	Holmes and Gay (1909) Stafford (1913)

¹ Some authors use *Cubaris* as a synonym for *Armadillo*, whereas others maintain that the two genera are distinct. The generic name *Armadillo*, although invalid by all rules of nomenclature, stubbornly persists because of long usage and the difficulty of finding a substitute without compounding the confusion. Hence, Jackson (1933), after reviewing the tangled nomenclature, advocates that *Armadillo* be "whitewashed of its past" and retained as a valid genus distinct from *Cubaris*.

² *Ligia* has priority over the commonly used *Ligyda* (Jackson, 1922).

³ *Ligidium hypnorum* (Cuvier) is a European species (Jackson, 1923). Richardson (1905, p. 686) gives California as one of its localities but remarks, "I have never seen any specimens of this species."

⁴ *Porcellio formosus* Stuxberg has not been reported from California since Stuxberg's original description (1875) of the species from specimens from San Francisco and San Pedro. I have reviewed the status of this species in a previous paper (Miller, 1936).

ARTIFICIAL KEY TO THE TERRESTRIAL ISOPODS
OF THE SAN FRANCISCO BAY REGION

1. Body contractile into a perfect ball; terminal segment of abdomen short, broad, and not exceeded by the short, flattened uropods. 15
- 1'. Body not contractile into a ball; terminal segment of abdomen usually triangulate, and exceeded by the styliiform, outer branch of uropod (see fig. 1) 2
2. Flagellum of second antennae (first rudimentary in all Oniscoidea) multiarticulate; head without lateral lobes, front broadly rounded (see fig. 1) 3
- 2'. Flagellum of second antennae with less than five articles; head with lateral lobes (sometimes small) 6

8. Uropoda with process on basal article at inner distal angle for articulation with endopodite, which is noticeably longer than exopodite; last segment of abdomen with lateral parts obsolete. 4
- 3'. Uropoda without process at inner distal margin; branches of uropods of about equal length; last segment of abdomen with lateral parts well developed (see fig. 1) . . . 5
4. Surface of body smooth and shiny. *Ligidium gracilis**
- 4'. Surface of body rough and scaly. *Ligidium latum*
5. Adult males with lateral plates (epimera) broadly expanded; eyes separated in front by distance equal to twice length of one eye; basal article of uropoda as broad as long 9
*Ligia pallasi**
- 5'. Epimera not so expanded; eyes separated in front by distance equal to length of one eye; basal article of uropoda longer than broad. *Ligia occidentalis**
6. Flagellum of antennae with less than four articles; basal article of uropods not laterally dilated to simulate epimera of fifth abdominal segment. 7
- 6'. Flagellum of four articles; basal article of uropods laterally dilated to simulate epimera of fifth abdominal segment. 14
7. Flagellum of two articles; external opercular branch of at least first two pleopods with tracheal organs. 8
- 7'. Flagellum of three articles; exopodites of pleopods without special respiratory organ 13
8. Abdomen abruptly narrower than thorax; frontal lobe of head absent, antero-lateral lobes small. *Metoponorthus pruinus**
- 8'. Abdomen not abruptly narrower than thorax; frontal lobe present, anterolateral lobes usually well developed. 9
9. Surface of body smooth or minutely granular. 10
- 9'. Surface of body roughly granulate or tuberculate
*Porcellio scaber scaber** and *P. scaber americanus**
10. Flagellum of antennae with first article less than one-half length of second
*Porcellio littorina**
- 10'. First article of flagellum of antennae not less than one-half length of the second. . . 11
11. Surface of body minutely granular; telson spatulate at tip
*Porcellio spinicornis occidentalis**
- 11'. Surface of body smooth and shiny; telson triangular at tip. 12
12. Articles of flagellum subequal; exopodite of first pair of pleopods of male rounded at tip *Porcellio formosus*
- 12'. First article of flagellum generally longer than second; exopodite of first pair of pleopods acuminate at tip. *Porcellio laevis**
13. Abdomen abruptly narrower than thorax; epimera small; body not very convex
*Philoscia richardsonae**
- 13'. Abdomen not abruptly narrower than thorax; epimera large with posterior angles acute; body very convex. *Alloniscus perconvexus**
14. Median and lateral lobes of head broadly truncate. *Actoniscus lindahli**
- 14'. Median lobe of head acute, lateral lobes rounded. *Actoniscus tuberculatus**
15. Outer branch of uropods small, inserted in middle of inner lateral margin of enlarged basal joint. *Cubaris californica*
- 15'. Outer branch of uropods large, lamellar, inserted at apex of basal joint
*Armadillidium vulgare**

ECOLOGICAL ASPECTS

The concept of "ecological niche" implies that each species occupies its own more or less restricted portion of an environment either because of structural or physiological adaptation to the conditions found therein, or because the biotic pressure of other species, competitors and predators, forces it into an available niche and keeps it there. Probably it is the combination of all these factors that restricts an animal, though it is possible that one of these may be the dominating or limiting factor. Grinnell and Storer (1924, p. 12) have said that "no two species well established in a region occupy precisely the same ecological space . . . If two species of the same ecological predilections are thrown into the same environment, one or the other will quickly disappear through the drastic process we call competitive replacement." Occupancy of practically the same place by two or more species does not necessarily mean that they have the same ecological niche, since they may so differ in food habits or in other respects as to be able to live harmoniously together.

The upper littoral zone in the San Francisco Bay region offers a number of ecological niches which are occupied by several species of terrestrial isopods. In this zone were found *Actoniscus lindahli*, *A. tuberculatus*, *Alloniscus perconvexus*, *Ligia occidentalis*, *L. pallasii*, *Philoscia richardsonae*, and *Porcellio littorina*. The two species of *Actoniscus* and *Porcellio littorina* were all found in the same place, namely, under rocks along sand and pebble beaches on the Alameda shores of San Francisco Bay. The other species are more definite and distinct in their habitat preferences. *Alloniscus perconvexus* burrows in the moist sand above high-tide line and may also be found under debris and washed-up seaweed along with the common beach amphipod, *Orchestoidea corniculata*, and, like it, is probably a scavenger. *Philoscia richardsonae* is a rather fragile species found in abundance along the margin of the bay, usually in grassy situations.

Ligia occidentalis is the common sea slater found under and among rocks on rocky beaches along the bay or ocean shore above high-tide line. It is a very important beach scavenger, feeding upon dead plant and animal material. It also feeds upon algae which it scrapes off rocks exposed at low tide. Like other species of *Ligia* (Nicholls, 1931), *L. occidentalis* is nocturnal and is active in the early morning and late afternoon.

Ligia pallasii replaces *L. occidentalis* on the northern Pacific coast, according to Johnson and Snook (1927). I have taken *L. pallasii* in large numbers from moist crevices in the faces of sea cliffs as far south as Montara, California, showing that the ranges of these two species overlap widely, although *L. pallasii* seems to prefer rocky sea cliffs, whereas *L. occidentalis* is found on rocky beaches.

Ligia pallasii exhibits marked sexual dimorphism in its body proportions (fig. 1), a fact not previously reported. The adult males are broad as a result of great lateral expansion of the lateral plates (epimera). The body index (length over width) averages 1.6. Females are much more narrow, with a body

index of 2.1, that is, over twice as long as broad. Young males are intermediate between adult males and females, and attain the expanded adult condition in a few molts. This statement is supported by observations on some young males which were measured during and after molting. Isopods molt by halves, with the posterior molt beginning at the fourth thoracic somite, followed, in the course of a few days, by the anterior molt. Thus, unlike some crabs, isopods remain active during the process of molting. Just after the posterior molt, one male specimen of *L. pallasii* measured 28 mm. in length and 17.5 mm. in width at the fourth thoracic segment, but only 16.0 mm. in width at the third

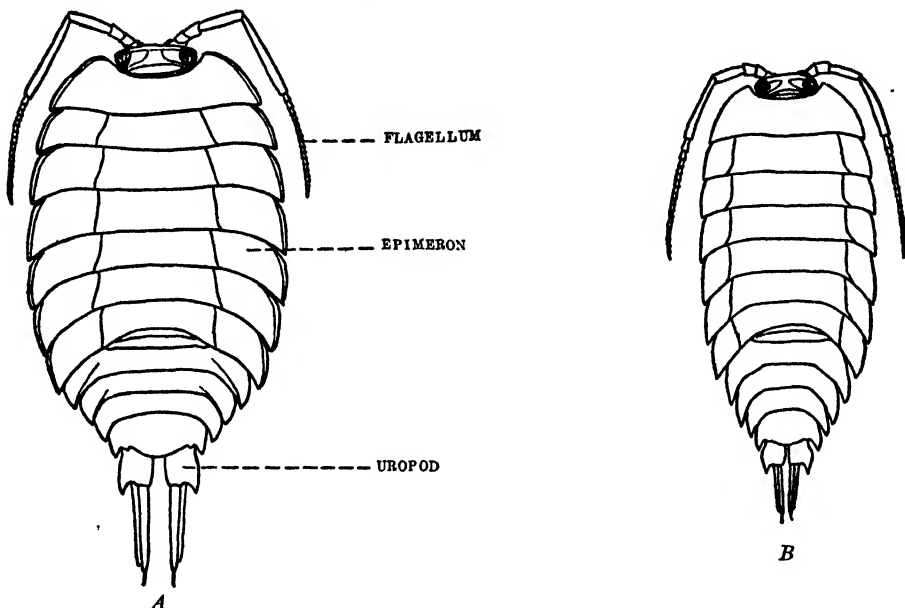


Fig. 1. Sexual dimorphism in *Ligia pallasii*. A, adult male; B, adult female. $\times 1\frac{1}{2}$.

(unmolted) thoracic somite. Two days later the anterior molt occurred, and the width at the third thoracic segment increased to 17.5 mm., without appreciable increase in the length of the body. This represents a change in body index from 1.75 to 1.6 in the course of a single molt, and indicates that the young males attain the expanded adult condition in the course of a few molts.

The expanded males often aggregate on the face of a cliff and form flat protective shields over the females and young males, which, because of their narrower bodies, are able to penetrate deeper into the crevices, thus escaping notice until the adult males are pulled away. Often the adult males cling to the backs of these females and young males. In Part II experimental evidence will be presented to show that this dimorphism has a survival value. No such dimorphism occurs in *Ligia occidentalis*, both male and female having about the same body proportions (body index 2.5).

The principal enemies of the two species of *Ligia* are birds, notably gulls, and shore crabs, especially *Pachygrapsus crassipes*, which lurks in crevices and catches the unwary *Ligias* in its chelipeds.

In northern California there are many streams running through canyons to the ocean from the summit of the coastal range of hills. Along the banks of these streams, densely shaded with California laurel and willow, may be found large numbers of a frail riparian species, *Ligidium gracilis*. This species is encountered under rotting logs, stones, and sometimes in the water, into which they jump for safety. Presumably, *Ligidium gracilis* feeds on the dead and decaying organic detritus along the banks. I have never found it along streams which dry up in summer.

The forms thus far discussed are probably native species. The more terrestrial species which are encountered upon leaving the streams are more widely distributed or cosmopolitan forms which have been introduced into gardens and cultivated areas. Here they are usually, but not always, more abundant than in the marginal areas, pastures, and regions of seminatural conditions into which they have migrated. This group of species is composed of *Armadillidium vulgare*, *Porcellio scaber scaber*, *P. s. americanus*, *P. laevis*, *P. spinicornis occidentalis*, and *Metoponorthus pruinus*. With the possible exception of *M. pruinus*, any of these species in sufficient numbers is capable of doing considerable damage to gardens and crops, and recently some of these have been suspected as the possible arthropod vector of the gizzard worm of poultry. *Armadillidium vulgare* has a particularly bad record as a hothouse pest (Richardson, 1905). *Porcellio scaber*, according to Essig (1926, p. 3), "eats holes in the stems of artichoke plants," one of the principal crops on the fog-swept peninsula south of San Francisco. That these species are capable of doing the damage ascribed to them is attested by the avidity and rapidity with which they consume carrot and potato slices and shoots in the laboratory. All these species are cannibalistic, at least in the laboratory. As a control to one of the experiments reported later, twenty specimens of *Metoponorthus pruinus* were placed in a humid, moist chamber without food. After half a year, only one of the twenty remained, the others having been successively devoured at their death.

These more terrestrial species also have their own ecological niche. *Porcellio scaber* is more abundant in moist situations along the coast and near streams. *P. laevis* and *P. spinicornis occidentalis* are found higher up in the hills than *P. scaber*, but rarely together. *Metoponorthus pruinus* is found more abundantly at still higher elevations. A few specimens were taken on Mount Diablo (about twenty miles inland) at an elevation of 2,500 feet, and they were abundant on Grizzly Peak in the Berkeley Hills at an elevation of about 1,500 feet. The range of *Armadillidium vulgare* overlaps that of all the others in this group, particularly that of *Porcellio laevis*. According to Essig (1926), *A. vulgare* has only recently been introduced into California. If so, it has spread rapidly and may displace in time some of the less hardy species with which it is now associated.

In table 1 the habitats or ecological niches are roughly arranged according to zones or altitudes, with approximate sea level at the bottom of the list. The species are purposely arranged in corresponding order, so that the table

TABLE 1
SUMMARY OF RECORDED COLLECTIONS, 1933-34

Ecological niche	Number of records	<i>Alloniscus perconezus</i>	<i>Actoniscus tuberculatus</i>	<i>Actoniscus lindahli</i>	<i>Porcellio littorina</i>	<i>Philoccia richardsonae</i>	<i>Ligia occi-dentalis</i>	<i>Ligia pallasi</i>	<i>Ligidium gracilis</i>	<i>Porcellio scaber</i>	<i>Porcellio spinicornis occidentalis</i>	<i>Porcellio laevis</i>	<i>Armadillidium vulgare</i>	<i>Neleponorthus prunosus</i>	Total: Number and percentage
High terrestrial zones															
{ High in Berkeley Hills (ca. 1000-2000 ft.)	7												11	462	473
{ Along bases of Berkeley Hills, west side	10										73	541	309	55	978
{ Near small creeks in Berkeley Hills, moist situations	4									218	6		95		100%
Zone															
{ Riparian; along banks of or in small creeks (Berkeley Hills; Moss Beach)	9								440	5					319
{ Rock cliffs along coast (Montara)	3							140	99%	1%					100%
{ Rock or boulder beach (Moss Beach; San Francisco Bay)	6														140
{ Salt-marsh grassland above high-tide line (San Francisco Bay)	2														100%
{ Rocky situations on pebble or sand beach, bay shore	2		18	25	12										250
{ Sandy beach (Moss Beach)	1	10	29%	38%	18%	10									100%
{ Total.....	10	18	25	12	260	440	140	440	223	79	541	425	517	

Upper littoral zone

graphically illustrates the sort of distribution encountered. The arrangement of habitats also corresponds, in general, to their moistness, except that the riparian habitat occupied by *Ligidium gracilis* is undoubtedly more moist than any of the others listed below it. Also, it is questionable whether the summits or higher elevations are drier than regions farther down the slope; the crests of the coastal range of hills are longer blanketed with fog, and there is more condensation and precipitation there than below, as indicated by the heavier growth of vegetation, as well as by meteorological records. Consequently, if the arrangement of species in the list were to be made on the basis of moisture of habitat, the following order would result: *Ligidium* > *Ligia* = *Alloniscus* = *Actoniscus* = *Philoscia* > *Metoponorthus* (?) > *Porcellio* = *Armadillidium*. This arrangement of genera in relation to the moisture of their habitat is based on very general observation. It must be emphasized that measures of the climatic conditions *where animals live* are needed. Ordinary climatological data are not adequate in studying the physical conditions of habitats, especially those of small animals which spend most of their time under objects such as rocks or logs, where special or microclimatic conditions obtain which may differ markedly from the climatic conditions of the general atmosphere.

The various species of isopods are not equally available at all seasons. Whereas those in the upper littoral zone and riparian zones can be collected at almost any time, the more terrestrial species are hard to find in natural conditions during the dry summer season. Species which are difficult to find in the hills during the dry months, however, may be found there in large numbers following the first heavy winter rains. I have not determined to my complete satisfaction just where these isopods go during the dry months, but I have some evidence to indicate that they migrate down deep cracks in the earth, into burrows of other animals, or into regions of greater moisture, and estivate in large aggregations. In August, I accidentally happened upon a large aggregation of *Armadillidium vulgare* in a shaded ravine, and from a plot one yard square by two inches deep I collected seventy-five males and sixty females.

Certain studies on the respiratory system of land isopods (reviewed by Mödinger, 1931) have indicated that there is a general correlation between structure and function and habitat. The respiratory system of isopods is found in the platelike branches of the biramous abdominal appendages. In aquatic forms and in some land forms, these thin lamellar branches serve as gills. Obviously, in order to function in respiration, the branchial plates must be kept moist, and land forms employing this method of respiration are limited to damp situations. In other land forms, a treelike branching system of minute air tubes, or tracheae, bathed in blood, ramifies through each exopodite of the first two pairs of pleopods, or, sometimes, of all five pairs. The trunk of each tracheal tree opens separately to the outside by means of a pore on the dorsal surface of the exopodite. It is possible that these tracheal organs are assisted in respiration by branchial plates on the more posterior pleopods.

The presence of these tracheal organs (usually indicated by whitish areas, the "white bodies" of early authors) enables their possessors to live in drier situations than those forms without them. On the basis of degree of development of the respiratory organs, Mödinger (*op. cit.*) divides the land isopods into four groups. The first group, composed of *Ligia* and *Ligidium*, he terms "amphibious," since they live close to water and may often jump into the water and remain submerged for some time. They breathe by means of gills and possess no tracheal organs. The family Trichoniscidae constitutes the second group, which is very like the first group except that the exopodite is used only as an operculum, and respiration takes place through the branchial endopodite. These forms are likewise confined to damp places. The third group is made up of the genera *Oniscus* and *Philoscia* (to which may be added the genus *Alloniscus*), which also breathe by means of gills, but which have special air chambers at the edges of the exopodites. In the fourth group he places *Cylisticus*, *Metoponorthus*, *Porcellio*, and *Armadillidium*, in which tracheal organs are developed. In *Metoponorthus*, tracheal organs are present in all five pairs of pleopods, but they are not so complex as those in *Porcellio* and *Armadillidium*, in which they are found only in the exopodites of the first two pairs of pleopods.

Mödinger considers the respiratory organs of *Armadillidium vulgare* to be the best developed, and that species to be the best adapted to life on land. Extirpation of the tracheate exopodites results in death in a short time, whereas removal of the exopodites in nontracheate forms causes no serious harm. In the fourth group there is also a strong development of the uropodial glands; which supposedly serve as an accessory to the respiratory system in that their secretion forms a moist layer over the pleopods, thus protecting them from drying. In aquatic isopods these glands are lacking, and in "amphibious" forms they are only weakly developed. *Ligia baudiniana*, according to Barnes (1932), helps to moisten its gills by bringing the uropodial spines together and dipping their ends into the water, which then runs up the spines and onto the gills by capillary action. This interesting reaction may also be employed by other isopods with the prerequisite uropodial structure, notably members of the family Ligiidae.

Applying the foregoing considerations to the species in the San Francisco Bay region, the genera may be arranged in order of increasing development of the respiratory system as follows: *Ligidium* = *Ligia* < *Alloniscus* = *Actoniscus* = *Philoscia* < *Metoponorthus* < *Porcellio* < *Armadillidium*. This order corresponds closely to the distributional series previously given (p. 121), and roughly to the altitudinal series given in table 1. In general, it is safe to say that the species with the more highly developed respiratory systems, including associated moisture-conserving devices, live farther from the water, in drier situations.

Associated with the more terrestrial isopods in their usual secluded habitats under rocks are many other animals, several of which prey upon them and are undoubtedly factors affecting their distribution and abundance. Among the

known vertebrate predators there are at least two species of salamanders, *Batrachoseps attenuatus* and *Aneides lugubris*, and several species of reptiles, birds, and insectivores. Both species of salamanders kept in the laboratory fed upon isopods. *B. attenuatus*, because of its small size, is able to eat only young or small specimens, whereas the large *A. lugubris*, which relishes isopods, can dispose even of large specimens of *Ligia*. On a number of occasions, however, I have seen large specimens of *Armadillidium vulgare* escape because of their ability to roll up into a ball which the salamander was apparently unable to swallow. The blue-tailed skink, *Plestiodon skiltonianum*, kept in the laboratory without other food, will dispose of an occasional isopod, and it is probable that other fairly common lizards, notably species of *Gerrhonotus* and *Sceloporus*, prey upon isopods. Birds in captivity eat isopods readily and probably feed upon them extensively in nature. Dr. A. H. Miller has observed flickers overturning rocks in the hills and feeding on the animals uncovered, which undoubtedly included isopods. Insectivores probably account for a good share of the isopods. Among the invertebrates, the black widow spider, *Latrodectes mactans*, and various species of centipedes are the principal predators of isopods.

Having to contend with all these predators and with a rigorous environment, as well as with possible cannibalism, isopods lead a precarious existence. To compensate in part for this high "environmental resistance" (Chapman, 1931), the "biotic," or reproductive, potential is also high. The number of young produced is not large; but the developing young are protected from some of the vicissitudes of the environment by being carried by the female in a brood pouch, or marsupium, formed by the overlapping of plates which arise from the bases of several pairs of walking legs. In the genus *Porcellio* an average of thirty-five young are found in the brood pouch. In *Ligidium gracilis* the average is twenty-three. My records do not indicate whether or not each female has more than one brood a year. Females carrying young in all stages of development are more abundant during February, March, and April, but they are also found in January, May, and June, coincident with the rainy season. Of the sixty females of *Armadillidium vulgare* previously mentioned as taken in an aggregation in August, not one was carrying young.

CONCLUSIONS

The foregoing discussion may be summarized as follows:

1. Each species of isopod may be assigned to its own particular place, or niche, in the environment. The ecological niches here investigated are grouped as follows: (a) upper littoral zone, in which are found species of the genera *Ligia*, *Philoscia*, *Alloniscus*, and *Actoniscus*, and *Porcellio littorina*; (b) the riparian zone, with *Ligidium gracilis* and an occasional *Porcellio scaber*; and (c) the higher and drier terrestrial zones, occupied by more widely distributed or cosmopolitan species, namely, *Metoponorthus pruinosus*, *Porcellio scaber*, *P. spinicornis occidentalis*, *P. laevis*, and *Armadillidium vulgare*.

2. The various species are more or less obviously adapted to their habitats with respect to their respiratory systems and water-conserving devices. There is a close inverse correlation between degree of evolution of the respiratory system and associated structures and moisture of habitat (see p. 122).

3. Sexual dimorphism is here reported in *Ligia pallasii*.

4. The food habits of various species are described. Some species are beneficial to man as scavengers, some are harmful, particularly as garden pests, and some are of no economic importance. Cannibalism is reported in animals kept in the laboratory, and it is suspected in nature.

5. All species are subjected to a high biotic pressure from predators, to which they respond with a fairly high reproductive potential, including protection of the developing young in a brood pouch.

6. There is a seasonal periodicity in breeding and other activity correlated with the incidence of rainfall. During the long dry season, the more terrestrial species are believed to migrate to the moistest place available and to estivate in aggregations.

PART II. EXPERIMENTAL STUDIES

The experiments were of two types: one group designed to test the ability of several species of terrestrial isopods to discriminate between humidities when offered a choice in a humidity gradient; a second group designed to determine the survival ability of each species under various combinations of constant humidity and temperature. Some species could not be collected in sufficient numbers and were therefore not used in some or all of the experiments.

HUMIDITY-GRADIENT EXPERIMENTS

The problem of these experiments was to determine the reactions of several species of terrestrial isopods to humidity gradients.

After considerable experimentation with various types of humidity gradients, a satisfactory apparatus was devised consisting of six cylindrical chambers 9 cm. in diameter and 6½ cm. deep. These were drilled in a circle through a block of wood 28 by 25 by 6½ cm., with partitions 1 cm. thick between chambers. Each chamber was connected to the two adjacent by means of tunnels 1½ cm. square cut out of the base of the partitions before the wooden bottom was nailed on. The entrances to these tunnels were provided with baffle plates and sliding doors which could be operated from the outside without disturbing conditions within the chambers. Each chamber was equipped with a hinged celluloid cover which could be quickly sealed in place with adhesive tape and paraffin. Two diametrically opposed chambers were further provided with glass tubes through which the animals could be introduced into the apparatus without disturbing the conditions within. The chambers were connected in a circular, rather than in the usual linear, order, after experiments with a gradient box of the latter type had proven unsatisfactory. With the chambers in linear series the animals in the control box became evenly divided between the two end chambers, which differed from the others only in having one exit in-

stead of two, so that the isopods traveling round the edges of the chambers in both directions collided and piled up. With the chambers connected in a circle, however, each chamber has two exits and isopods in control boxes of this type remain approximately equally divided among the six chambers (see table 2).

The method of establishing a humidity gradient was suggested by the work of Williams (1932), who placed a dish of CaCl_2 in one end of a five-chambered gradient apparatus and a dish of water in the other end, establishing a gradient based on diffusion as follows (reading from dry chamber to wet): 15–20 per cent, 30–35 per cent, 55–60 per cent, 80–85 per cent, 100 per cent. Since sulphuric acid of a given concentration will maintain a given humidity in a closed chamber (see table 5), it was decided to establish the gradient by placing stender dishes of acid of different concentrations appropriate to the gradient desired in each of the chambers. This was on the assumption that the acid in a gradient chamber would maintain nearly the same humidity as in an isolated chamber, provided the rate of diffusion between chambers was small and differences between adjacent chambers were not too large. It was thought that the small size of the communicating tunnels, the baffle plates, and the stender dishes in the chambers would impede the progress of diffusing water vapor, and thus keep the rate of diffusion low. In order that the expected humidity gradient might prevail at least at the beginning of the experiment, each chamber was closed off an hour or two before each experiment by means of the sliding doors to allow the acid to come into humidity equilibrium with the air. No instrument was made to measure the actual humidity in the chambers, but the specific gravity of the acid from each chamber was measured after each experiment. It was found that the more concentrated acids in the drier chambers decreased in specific gravity by taking on water, whereas the less concentrated acids in the moisture chambers increased slightly by giving off water; but there never was enough variation to alter the humidity in any chamber more than 5 per cent from the theoretical.

With the six chambers of the gradient box arranged in a circle, it was advisable to establish either a double gradient composed of four chambers, each with the two end chambers in common, or a double gradient of three chambers, each connected in parallel at the extremes. If, for example, it is wished to establish a gradient ranging from zero to 100 per cent, the water and acids could be arranged either as in diagram *A* in figure 2, or as in diagram *B*. It is clear that *A* is the better arrangement; but *B* was also used, especially when the range of the gradient was not great and the steps between chambers were correspondingly smaller. A number of other combinations also are possible.

At the beginning of an experiment, the doors between the chambers were opened, and twenty animals were introduced into the apparatus in two groups of ten each through the glass chutes in the covers of two diametrically opposed chambers. Experiments were carried on in the dark because, in preliminary tests with evenly illuminated chambers, a slight tendency was evidenced for the animals (which are negatively phototropic) to remain in the shaded tunnels between chambers. Readings were made by red light. The animals in each

chamber of the control and experimental boxes were counted every few minutes over a period of two or three hours. The numbers for each chamber over the total period of time were totaled, and the average percentage of preference calculated. Since there were no significant unilateral differences in chambers with the same humidities, the results from these experiments were lumped together in the computations. As the actual tabulations are long, only the average percentage preferences for each experiment are given in the tables of results. These percentages do not show, however, that the animals are rather active at first but become more quiescent as the experiment progresses, and that the trend, soon evident, becomes more and more marked.

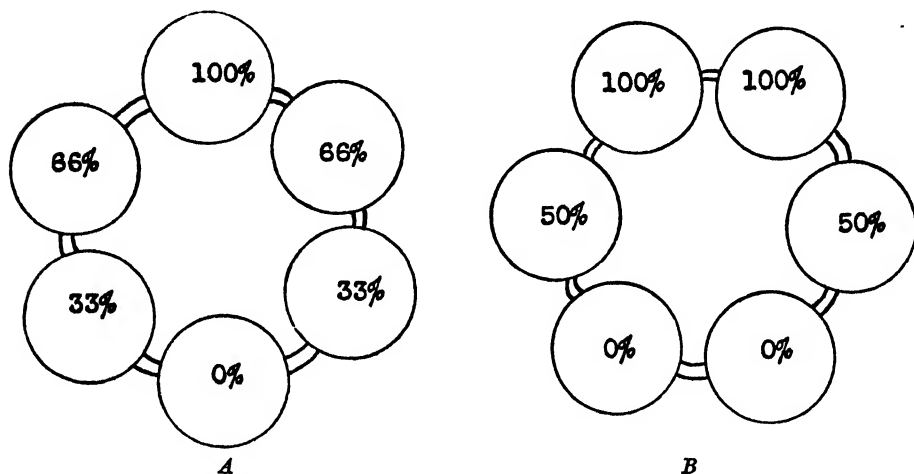


Fig. 2. Diagrams A and B. Gradient ranging from zero to 100 per cent.

Unfortunately, only four species were studied, *Armadillidium vulgare*, *Porcellio laevis*, *P. spinicornis occidentalis*, and *Metoponorthus pruinosis*, all of which belong to the more terrestrial group. A few experiments were made using specimens of *A. vulgare* with the antennae removed.

The results of the experiments are summarized in tables 2-4.

The following conclusions may be drawn from the gradient experiments.

1. The results definitely show that the four species of isopods tested reacted to the gradients, always aggregating in greater numbers in the chambers with the highest humidity in the gradient used; whereas in the controls there were no preferences for any chamber.

2. They also indicate that the preferences were progressively less marked in gradients with shorter range and at the dry end of the scale. For instance, the preferences of *Armadillidium vulgare* for the 100-per cent relative humidity chambers in three different combinations were 81.8, 71.6, and 66.6 per cent, whereas the preferences when the moistest chambers were 75 and 50 per cent were 47.7 and 39.3 (and 44.0) per cent, respectively. By decreasing the range of the gradients, it might be possible to determine a limen, or threshold value, for ability to discriminate.

TABLE 2
HUMIDITY PREFERENCE IN PERCENTAGES

Species	Experimental box					Control box						
	Gradient (percentage of relative humidity)					Percentage of relative humidity						
	0%	33%	66%	100%		100%	100%	100%	100%	100%	100%	100%
<i>Armadillidium vulgare</i>	4.5	5.1	8.6	81.8		15.6 { 16.5	15.2 17.6	15.6 11.9	15.9 18.5	17.3 17.0	19.9 18.2	
<i>A. vulgare</i> (without antennae)....	2.4	5.0	9.7	82.8		17.4 { 17.5	20.5 16.9	16.3 15.7	14.0 15.7	15.4 16.1	16.3 19.3	
<i>Porcellio laevis</i>	2.0	3.7	8.3	85.8		15.4 { 13.8	14.8 19.0	11.9 19.6	17.3 14.3	22.3 14.3	18.0 18.7	
<i>P. spinicornis occidentalis</i>	9.6	16.4	20.1	53.4		17.4 { 16.2	15.1 12.1	14.2 18.2	16.9 18.5	19.8 16.5	16.3 18.2	
<i>Metoponorthus prunosus</i>	1.5	6.2	13.5	78.7								

TABLE 3
HUMIDITY PREFERENCE IN PERCENTAGES*

Species	Experimental box										
	Gradients (percentage of relative humidity)										
	0%	25%	50%	75%	50%	75%	100%	0%	25%	50%	75%
<i>Armadillidium vulgare</i>	{ 28.8 23.2	{ 31.6 32.8	{ 39.3 44.0*	19.4	32.8	47.7	66.6	—	—	—	—
<i>A. vulgare</i> (without antennae).....	23.2	32.8	44.0	—	—	—	—	—	—	—	—
<i>Porcellio laevis</i>	20.6	31.5	47.8	—	—	—	—	—	—	—	—
<i>P. spinicornis occidentalis</i> ...	—	—	—	—	—	—	—	18.0	18.1	20.1	43.7

3. The reactions of *Porcellio spinicornis occidentalis* to the gradients were much less marked than in the other three species.

4. Sensitivity to moisture in *Armadillidium vulgare*, at least, is not exclusively dependent on receptors in the antennae, since specimens with antennae removed reacted practically the same as uninjured specimens.

5. It seems probable that the aggregation of the animals in the moistest chamber was brought about by conditions set up internally by loss of water

TABLE 4
HUMIDITY PREFERENCE IN PERCENTAGES*

Species	Experimental box					
	Gradients (percentage of relative humidity)					
	0%	33%	66%	33%	66%	100%
<i>Armadillidium vulgare</i>	—	—	—	9.8	18.5	71.6
<i>Porcellio laevis</i>	—	—	—	2.0	12.2	85.7
<i>P. spinicornis occidentalis</i>	31.1	26.0	42.9	—	—	—

* Controls as in table 2.

and/or drying of the respiratory membranes, resulting in greater random activity, eventually bringing the animal into regions of greater comfort—specifically, the moister chamber.

The ecological implications of these results will be considered later.

SURVIVAL EXPERIMENTS

The problem of these experiments was to determine the survival abilities of eight species of terrestrial isopods at various constant humidities and temperatures.

Constant humidities were maintained most satisfactorily by using sulphuric-acid solutions of proper concentration (see table 5).

In the first series of experiments a constant temperature of $20^{\circ} \pm 1^{\circ}$ C. was maintained in order to ascertain the effect of humidity alone. In later series, constant temperatures of 25° , 30° , and 35° C. were used in combination with various humidities so that the relative effects of temperature and humidity could be compared.

For experimental and control chambers, desiccators were used in the first series, whereas in later series with higher temperatures a small constant-temperature box was sealed air-tight and converted into a desiccator by installation of a large acid container on the floor of the chamber. Since it was necessary to probe the animals to determine the death point, the chambers were equipped with wire probes which could be manipulated from the outside without admitting air.

In order to establish an equilibrium of the required humidity, the proper concentrations of acid were allowed to stand in the chambers for several hours before the experiment. The humidity was checked before and after each ex-

periment with a dew-point thermometer, and after each experiment the specific gravity of the acid was again tested with a hydrometer. There was no appreciable variation in either the humidity or the specific gravity of the acid.

From each species of isopod, twenty adult individuals, of both sexes, were selected for each unit of the experiment (a given combination of humidity and temperature), with the exception of *Ligia pallasii*, from which experimental samples were usually composed of fifteen individuals assorted by sex in order to determine whether the marked sexual dimorphism, previously

TABLE 5
SATURATION DEFICITS AT DIFFERENT TEMPERATURES IN RELATION TO PERCENTAGES
OF RELATIVE ATMOSPHERIC HUMIDITY

Specific gravity	Percentage of H ₂ SO ₄ in aqueous solution	Approximate relative humidity, in percentages	Saturation deficit in mm. Hg.			
			At 20° C.	At 25° C.	At 30° C.	At 35° C.
1.00	0.00	100.0	0.0	0.0	0.0	0.0
1.09	12.99	95.0	0.9	1.2	1.6	2.2
1.14	19.61	90.0	1.8	2.4	3.2	4.3
1.23	31.11	75.0	4.4	6.0	8.0	10.7
1.27	35.71	66.0	6.0	8.2	10.9	14.6
1.29	38.03	60.0	6.8	9.3	12.4	16.6
1.340	43.6	50.0	8.7	11.9	15.8	21.1
1.361	46.0	45.0	9.7	13.1	17.0	23.2
1.417	52.4	33.0	11.5	15.8	21.0	28.1
1.438	54.0	30.0	12.4	16.8	22.0	29.8
1.459	56.0	25.0	13.1	17.8	23.4	31.7
1.479	58.0	21.5	13.9	18.7	24.3	33.1
1.524	62.0	15.0	14.8	20.1	26.5	35.7
1.569	66.0	10.5	15.6	21.3	28.0	37.8
1.840	100.0	0.0	17.3	23.8	31.5	42.3

noted, had any differential survival value. At first, experiments were tried with samples composed of several species, but the results were too often complicated by the fact that dying individuals of one species were devoured by members of the hardier species, and even occasionally by members of their own species. In later experiments with assortment by species, cannibalism was negligible. Generally, freshly molting individuals and females with young in the brood pouch were excluded. Of the 93 units of the experiment, 56 were repeated at least once, so that the majority of the mean survival times were calculated from samples of 40 and 60 individuals (see table 6).

At the beginning of a unit of the experiment, the animals were dropped into the chamber through a glass tube in order to disturb the conditions within the chamber as little as possible. The starting time was recorded and readings were taken at appropriate intervals to determine the time of death. An animal was judged to be dead when probing failed to evoke any movement, especially of the antennae. It was feared that the death feigning, especially of *Porcellio*, might invalidate this procedure, but it was found that the animals abandoned

this mode of behavior long before actual death. A relative humidity of 100 per cent was maintained in the control chambers.

Results of the experiments are summarized in table 6, and in figure 3 by the survival curves for the various species. It was neither possible nor necessary to determine exact survival times of the control animals kept in saturated chambers, since they far outlived the experimentals (some of the controls were kept alive for months), and since starvation, cannibalism, and other factors probably contributed more to their death than did the conditions of moisture and temperature.

From the series of survival experiments the following conclusions may be deduced:

1. It is evident that the optimum relative humidity for survival is close to 100 per cent, or a saturation deficit of zero.

2. As humidity decreases, survival times for each species become progressively shorter, and even at moderate humidities survival is only a matter of a few hours. The results are therefore not complicated by starvation.

3. Survival is not inversely proportional to saturation deficit, as some investigators have found for other arthropods (see Buxton, 1932), but there is a rather precise relationship between the two variables which warrants formulation. If we plot mean survival times logarithmically against saturation deficit, we obtain the best linear alignment of the data points for all species except *Ligia occidentalis*, and hence the best expression of the relationship is an exponential equation of the form

$$T = a \cdot e^{-ms}$$

where T is mean survival time (in minutes), a and m are constants, and S is saturation deficit (in mm. Hg.). This relationship obviously does not hold for extremely low (optimum) saturation deficits, and, occasionally, also breaks down at extremely high deficits. The constants a and m for each species, calculated by the method of moments, are given in table 7.

4. The data for *Ligia occidentalis* gave the best linear alignment when both mean survival time and saturation deficit were plotted logarithmically, and hence could best be fitted by a power function of the form

$$T = a \cdot S^{-b}$$

Calculating the constants a and b by the method of moments we get

$$T = 10,470 \cdot S^{-0.9067}$$

Again the relationship breaks down at the extremely low optimum saturation deficits. It also was found that a fairly good straight line could be obtained by plotting the reciprocal of survival time against saturation deficit, indicating that survival may be inversely proportional to saturation deficit. In the equation above the proximity of the exponent to negative unity indicates the same thing. We do not have enough data for *Ligia pallasii* to formulate any equations, but we can predict from the information at hand and from the

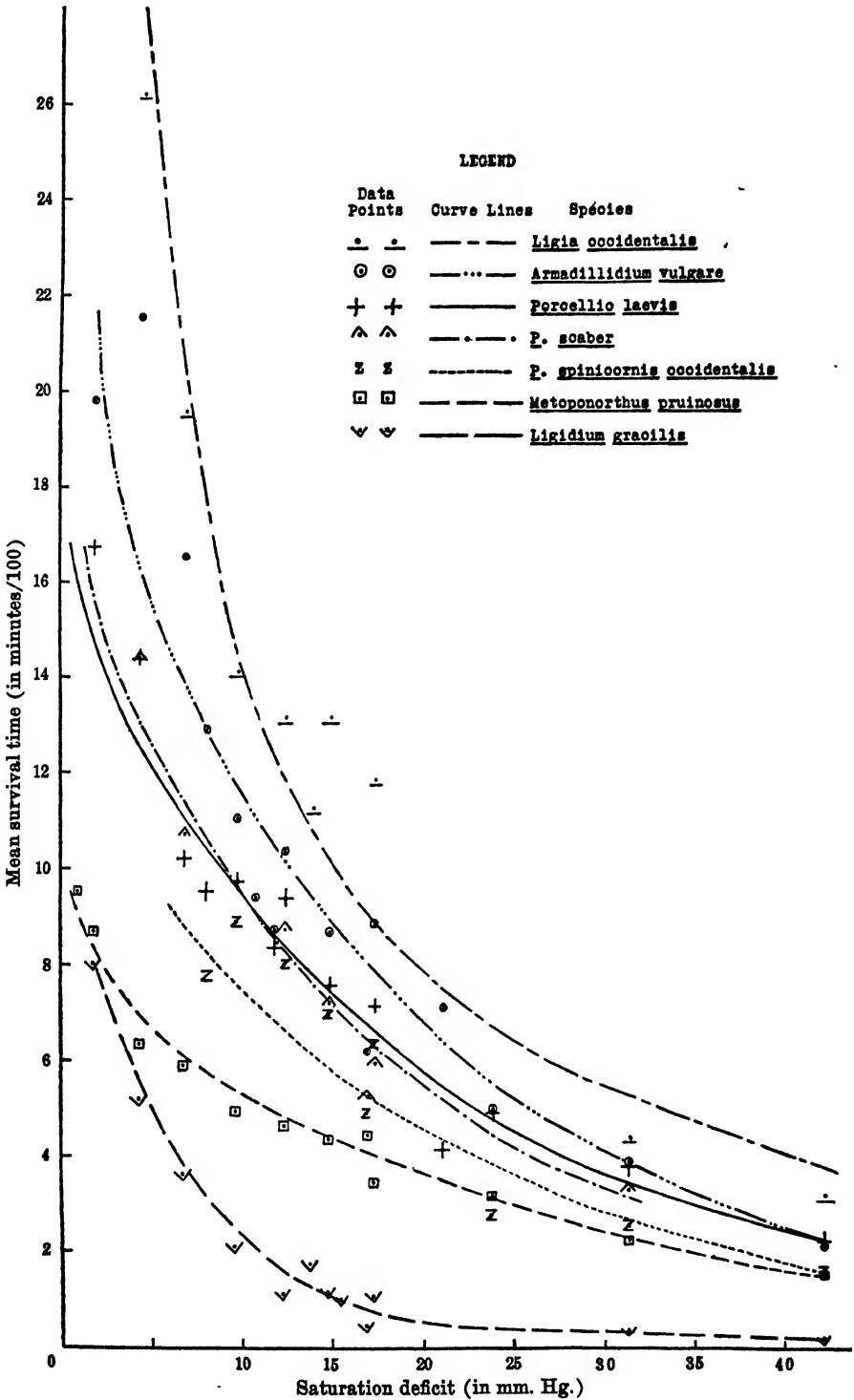


Fig. 3. Survival curves.

similarity of equations for the three species within the genus *Porcellio* that any mathematical expression evolved for *L. pallasii* would have a form similar to that of *L. occidentalis*.

In the foregoing analysis the temperature variable has been disregarded in order to get a simple approximation of the relationship between survival and humidity. This procedure is justifiable because, as will be seen, the differences between mean survival times for any species at identical saturation deficits produced at different temperatures are small, and sometimes not significant. Also, the points on the graph representing the higher saturation deficits, requiring higher temperatures to produce them, seemed to be extensions of the curve for survival at lower deficits and temperatures. When more data at lower deficits and higher temperatures are available, it may be possible and necessary to make a finer analysis including the temperature variable.

TABLE 7
CONSTANTS USED IN DETERMINING THE SURVIVAL TIMES OF ISOPODS
UNDER DIFFERENT DEGREES OF HUMIDITY

Species	a	m
<i>Porcellio laevis</i>	1529	0 0488
<i>P. scaber</i>	1603	0 0534
<i>P. spinicorns occidentalis</i>	1254	0 0518
<i>Armadillidium vulgare</i>	2071	0 0560
<i>Metoponorthus prunosus</i>	836	0 0420
<i>Ligidium gracilis</i>	1001	0 1505

5. Increase in temperature within the range used (20°–35° C.), with humidity constant, results in shorter survival times. In order to interpret correctly the relative effects of humidity and temperature, choice must be made between the expressions which we have used for humidity, namely, saturation deficit and relative humidity, since it makes a great difference in analyzing the effects of temperature on survival whether humidity is kept constant in terms of saturation deficit or of relative humidity. Decision should be made on the basis of better biological meaning. It seems reasonable to assume from the nature of the experiment and its results that death, however brought about, is conditioned by loss of water through evaporation. If so, then saturation deficit has a better biological significance, as Buxton (1932) has pointed out, because evaporation in a physical system, at least, is proportional to saturation deficit according to Dalton's Law, and not, therefore, to relative humidity.

The better significance of saturation deficit becomes more obvious if the effects of different temperatures on survival of a given species are compared, keeping the humidity constant (1) in terms of relative humidity and (2) in terms of saturation deficit. For instance, for *Armadillidium vulgare* (see table 6) the mean survival times at 0 per cent, with relative humidity at 20°, 25°, 30°, and 35° C., are 882, 498, 390, and 207 minutes, respectively. Now these

temperatures are well within the range of those encountered in nature, and yet, with each rise of 5°C. , the survival time is lowered approximately 40–50 per cent. The average temperature coefficient, Q_{10} , in this instance, is 2.7. There is no apparent reason or explanation for such a large temperature effect. On the other hand, the mean survival times for the same four temperatures and a saturation deficit of 17.4 mm. Hg. are 882, 700, 613, and 783 minutes, respectively (last three values obtained by interpolation), and Q_{10} is about 1.0. The differences here are much smaller, some of them not statistically significant, and not always in the same direction, although, in general, we observe a decrease in survival time with increase in temperature. Again, considering the range of temperature used, these results are much more plausible. The slight decreases in survival time with increased temperature can be attributed to the effect of temperature in speeding up metabolism, with consequent increase in production of metabolic water and its subsequent loss through evaporation.

It may be concluded, therefore, that saturation deficit is the better expression for humidity, and as such it is gaining in favor and usage. If mean survival times at different temperatures with humidity constant in terms of saturation deficit are compared, the differences are seen to be small and, in comparison with the effects of humidity on survival, the effects of temperature within ordinary ranges are almost negligible.

6. The differences in ability of species to survive under the same humidity conditions are graphically illustrated in the divergences of the survival curves (fig. 3). These differences are greater in the intermediate regions of the curves than at the two extremes, and are there statistically significant, being over four times greater than their probable errors. As the optimum saturation deficit of zero is approached, and to a lesser extent the opposite extreme, the probable errors become relatively greater and the specific differences in length of life become smaller and statistically less important, or even insignificant. Nevertheless, the differences are always in the same direction, except for *Porcellio laevis* and *P. scaber*, whose survival curves cross at a saturation deficit of about 10 mm. Hg. We may consider these differences as physiological characters separating species and genera; as in other taxonomic characters, the differences are greater between genera than between species in the same genus. Arranging the species studied on the basis of these differences and in order of increasing ability to survive the intermediate range of humidities of the experiments, the following survival series is obtained: *Ligidium gracilis* < *Metoponorthus pruinus* < *Porcellio spinicornis occidentalis* < *P. scaber* \leq *P. laevis* < *Armadillidium vulgare* < *Ligia occidentalis* < *L. pallasii*. (See p. 135 for further discussion of this series.)

7. Another factor relating to the viability of these animals under conditions of low humidity is their so-called "bunching" reaction (Allee, 1926). Under all but the highest humidities, and sometimes even then, the isopods aggregated to form tight bunches and remained thus until shortly before the death of the first individual, when the bunch would break up. In respect to *Ligi-*

dium gracilis, the bunches invariably scattered quickly, about fifteen minutes before the death of the first individual in the first series of the experiment, and in shorter times in the other series. It would be interesting to determine experimentally the efficacy of the bunching reaction in prolonging average life.

A pertinent case in point is manifest in comparing the results for the two sexes of *Ligia pallasii*, although there is only one experiment at a saturation deficit of 17.4 mm. Hg. and at 20° C. for comparison. With this combination of moisture and temperature, the expanded adult males (see fig. 1) formed a disk-like bunch in which about three-fourths of the individuals were shielded beneath the surface of the mass. The mean survival time of the group was 5007 ± 307 minutes, or nearly twice that of a like number of the narrower females of the species (mean survival time 2400 ± 46 minutes), whose bunching was less compact and apparently not so protective.

DISCUSSION

We may now compare the results of the various phases of the study and discuss their ecological, physiological, and evolutionary implications.

A major part of the work may be summarized and brought into relation by comparing the four different series or arrangements of the species which have been made on the basis of similarities and differences in habitats, adaptations to habitats, and ability to survive under conditions of controlled humidity and temperature. These are as follows:

Habitat series

1. On the basis of increasing altitude and distance from the seashore:

Alloniscus = *Ligia* = *Actoniscus* = *Philoscia* < *Ligidium* = *Porcellio* = *Armadillidium* < *Metoponorthus*

2. On the basis of moisture of habitats:

Ligidium > *Ligia* = *Alloniscus* = *Actoniscus* = *Philoscia* > *Metoponorthus* (?) > *Porcellio* = *Armadillidium*

Structural adaptation series:

3. On the basis of increasing degree of evolution of respiratory system and associated devices for moisture conservation:

Ligidium = *Ligia* < *Alloniscus* = *Actoniscus* = *Philoscia* < *Metoponorthus* < *Porcellio* ≤ *Armadillidium*

Survival series

4. On the basis of increasing ability to survive suboptimal humidities:

Ligidium < *Metoponorthus* < *Porcellio* (*spiniornis occidentalis* < *scaber* ≤ *laevis*) < *Armadillidium* < *Ligia* (*occidentalis* < *pallasii*)

The correlations among the first three series have been discussed in Part I. It is evident that the survival series correlates well with the other three, especially with series 2 and 3, if the genus *Ligia* is left out of consideration. In other words, the species which survive longest under experimental low humidities in the laboratory are those which live in the drier habitats and have the best adapted respiratory systems, as would be expected. But the superior position of the genus *Ligia* in the survival series is grossly inconsistent with the low

degree of evolution of its respiratory system and the fact that it occupies one of the moistest habitats in nature. In order to explain the correlation and to reconcile the inconsistency, it is necessary to consider the possible causes of death in the survival experiments.

From the correlations established, we might have assumed that drying of the respiratory membranes was responsible for death, since those species with the better developed tracheal organs and other devices for protecting the respiratory membranes from desiccation were able to survive the conditions of the experiments longer than species with less adapted respiratory systems. In the genus *Ligia*, however, the respiratory system is essentially branchial, and uropodial glands are only weakly developed; yet the two species of *Ligia* were far superior to the others in their ability to survive suboptimal conditions. If we assume that death is caused by loss of water below some physiological vital level, as Numanoi (1934) has shown in *Ligia exotica*, and that water is lost mainly, if not exclusively, through the respiratory system, as Mellanby (1934) has suggested for insects, the correlation might still hold true even for *Ligia*, provided the lethal level of water loss is approximately the same for all species; the superior position of *Ligia* could then be explained on the basis of size. Species of *Ligia* are much larger than any of the others, and hence it would require relatively more time to reduce the water of the body to the lethal level. It is also possible that some water may be lost through the general body surface. Other things being equal, such a situation would also favor a larger animal with proportionately less surface to volume. The species other than *Ligia* are too nearly the same size, however, for a surface-to-volume ratio explanation of the observed differences in their survival ability. The correlation between survival and degree of evolution of the respiratory system and associated moisture-conserving devices has fundamental significance, whatever explanation we adopt for the cause of death.

The fact that in the experiments optimum humidity for survival was found to be a saturation deficit of zero, and that the species cannot long endure lower humidities, leads us to a consideration of the ecological effects of natural humidity conditions. Climatic records for various parts of the San Francisco Bay region show that the average relative humidity is between 60 and 75 per cent, that the humidity rises in the evening and through the night to between 90 and 100 per cent, as a rule, owing to fog, lower temperature, etc., and that during the day it generally falls to between 40 and 60 per cent. The mean survival times (table 6) of the various species at these relative humidities at any of the temperatures show that under humidities equivalent to average diurnal humidities most species died off within twenty-four hours, and even at 90 per cent relative humidity only the *Ligias* survived more than two days. Although the conditions of humidity in nature are not constant as they were in the experiments, the inevitable inference is that the species would die off in comparably short times if subjected only to general humidity conditions.

The question arising is, How can the species maintain themselves in nature if general humidity conditions are lethal in so short a time? Death is condi-

tioned by evaporation of water reducing the body water below some vital level and/or drying of the respiratory membranes. In order for the isopods to survive, evaporation must be kept at a minimum; lost water must be replaced. Cook and Scott (1932) have shown that termites replace lost body water with water derived from food, but are not able to absorb water from a saturated atmosphere, and that recovery may take place if the loss of body water has not been too great. It is undoubtedly true that isopods also extract considerable water from food to replace that lost. The excreta are rather dry, and practically all water is lost by evaporation from the respiratory system. I have already discussed the adaptations of the respiratory system in various isopods for water conservation, and it has been seen that, at best, they are not sufficient to cut down the rate of evaporation so that the animals could survive conditions of general humidity without food. Their only alternative is to find places with higher humidity or lower saturation deficit than the general atmosphere, with proportionately lower rate of evaporation.

It is presumed that the microhumidity conditions under boards, rocks, and other objects are more nearly saturated or optimum. Williams (1932) has shown that it requires very little soil moisture to maintain saturated conditions in the runways of subterranean termites, and this is undoubtedly true for conditions such as those in which isopods are found in nature. During the day, especially when atmospheric humidity is low, the negative phototropism, positive thigmotactic response, and the ability demonstrated in gradient experiments to select regions of greatest humidity, all assist in bringing the isopods into places of higher humidity. During the night, or whenever the general atmospheric humidity is more favorable, the isopods may emerge to forage, if necessary. Thus, they are able to circumvent in nature the experimentally demonstrated lethal effects of even moderately high humidities.

So, in contrast to the hosts of insects which have radiated into almost every conceivable terrestrial habitat, the isopod invaders of the land have been limited to undercover activities, and will remain thus restricted to places of high microhumidity until they can evolve a more satisfactory defense against desiccation. In terms of Leibig's Law of Minimum, atmospheric humidity is always near or below the limits of toleration, and hence it is the controlling and limiting factor in the ecology of isopods. The species which have become better adapted to withstand desiccation have been able to penetrate farther inland away from their ancestral home, the sea, but, at best, they are still transitional between aquatic and truly terrestrial life.

Before the migration from water to land could take place at all, a certain amount of structural and physiological adaptation, or preadaptation, must have existed. The possession of branchial pleopods which could serve the function of aerial respiration if kept moist offered the main point of departure. Even now, isopods of several marine tribes, living in the intertidal zone, are periodically exposed to semiterrestrial conditions when the tide goes out. The isopods living in the upper littoral zone have gone but little farther in evolution, and have, in a sense, carried the sea with them in their body fluids and

in the form of a thin film of water over their branchial plates. In addition, there are other features common to the order, or, at least, found in some aquatic forms, which the terrestrial isopods have taken with them and which have proved useful in the struggle for existence on land. Among these are certain behavior patterns, such as negative phototropism, positive thigmotaxis, and the bunching reaction; depressed body form, which enables them to crawl under objects more easily; the brood pouch in which the young are protected from some of the vicissitudes of the environment, particularly desiccation; molting by halves with retention of motility during the process; and other features of lesser utility.

Man has been a valuable, though unwitting, ally to the isopods not only in the widespread distribution of the more hardy species as stowaways in his various transports, but also in providing ample coverage in the form of débris about his habitations, watered gardens and farms, hothouses and nurseries; in short, favorable conditions for the maintenance of an isopod fauna. This is especially true in California, for the long dry season and the small amount of annual rainfall characteristic of this State are not favorable conditions for isopods and account for the taxonomic poverty of species of this region (Arcangeli, 1932; Miller, 1936). Most of the more terrestrial species are importations; hence their spotty distribution around centers of population and irrigated areas.

SUMMARY

The results of these comparative ecological studies on the terrestrial isopods of the San Francisco Bay region are reported in two parts. Part I deals with natural history and includes a check list of the terrestrial isopods of California, a key to the species of the Bay region, study and comparison of the habitats and adaptations of the various species to their habitats, and other ecological notes on biotic factors in the environment. Part II deals with laboratory experiments on (1) the reactions of the isopods to various humidity gradients, and (2) the effects of various combinations of humidity and temperature on survival of the different species. An attempt is made in the discussion to correlate and integrate the major phases of the study.

Humidity is considered to be the dominant and limiting factor in the environment of isopods, as is shown by the following:

1. Optimum humidity for survival is a saturation deficit of zero, and the species cannot long endure lower humidities. There is a precise relationship between survival and saturation deficit capable of expression in simple equations.

2. The isopods are predominantly found in places of high or optimum micro-humidity, and it is shown to be highly improbable that any species can maintain itself without access to such places.

3. There is a triple correlation between the species in moisture of their habitats, degree of evolution of their respiratory systems and associated water-conserving devices, and their respective abilities to survive experimentally produced low humidities.

4. There is a seasonal periodicity in breeding and other activity correlated with the incidence of rainfall.

5. The taxonomic poverty of species in California and their spotty distribution are associated with the long dry season and the small amount of rainfall characteristic of the State.

Temperature is considered to be of little ecological importance except as it influences humidity. Increase in temperature from 20° to 35° C., with saturation deficit constant, has very little effect on survival of animals in laboratory experiments in comparison with changes in humidity.

The isopods studied show several stages in the transition between aquatic and terrestrial life. The Oniscoidea cannot be considered to be successful invaders of the land, because they have not satisfactorily solved the related problems of water conservation and aerial respiration.

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**THE DEVELOPMENT OF
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WITH OBSERVATIONS ON THE ORIGIN
OF THE EXTRAEMBRYONIC COELOM
AND FOETAL MEMBRANES**

BY

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THE DEVELOPMENT OF THE EXTERNAL FORM OF THE RAT, WITH SOME OBSERVATIONS ON THE ORIGIN OF THE EXTRAEMBRYONIC COELOM AND FOETAL MEMBRANES

BY

J. A. LONG AND PAUL L. BURLINGAME

IN THE ABSENCE of any complete account of the development of the external form of the rat, and with the advance in methods of study in the past few years, it has seemed worth while to offer a series of illustrations depicting the establishment of the external features of this animal from their first appearance in the egg cylinder to late gestation. Our predecessors in the study of the rat (Fraser, 1882; Selenka, 1884; Cristiani, 1892; Duval, 1891; Robinson, 1892, 1904; Widakowich, 1909; Huber, 1915; Grosser, 1909, 1927) and of the mouse (Fraser, 1882; Selenka, 1883; Duval, 1891; Robinson, 1892, 1904; Jenkinson, 1900; Melissinos, 1907; Sobotta, 1895, 1902, 1911) have been concerned with the stages from cleavage to the first beginnings of the foetal membranes, including the origin of the three germ layers, the egg cylinder, "inversion of the germ layers," the coelom, implantation, and uterine changes. Only one of them, Widakowich, has considered stages up to about twelve days of gestation and has given figures of the exterior. Incidentally, Adelmann (1925) shows external form as far as the neural folds contribute to it. Since then (1927), a study of the stages involving the reversal of curvature was made in this laboratory by Pauline Barden and embodied in an unpublished master's thesis.

Recent papers by Harman and Prickett (1932, 1933) concern the external form of the guinea pig.

Although the intention was to deal only with the external form in this paper, the search for the earliest stages brought to light valuable material showing the origin of the extraembryonic coelom and foetal membranes, part of which is here presented, both by way of illustrating further the work of the early investigators and also to add to their accounts. For histological details the reader is referred to the papers of Robinson, Widakowich, Sobotta, and Huber, which contain excellent reviews and discussions.

For work on the later foetal membranes, which are shown only incidentally in this paper, mention must be made not only of the papers listed above, but also of those by Asai (1914), and especially by Everett (1935) for the placenta and yolk sac.

Our work has been aided by funds from the Board of Research, University of California, and by assistance provided by W.P.A. Project no. 4739.

AGE OF EMBRYOS

In a series of rat embryos of known copulation age prepared some years ago by the senior author it was found that the state of development did not conform to what was expected from the records. The same discrepancies are evident in the data published by Widakowich and by Huber and were observed also in embryos (carefully collected in this laboratory by Miss Barden and by F. Rutherford) removed from one horn of the uterus several hours after the embryos from the other horn were taken out by aseptic surgery. The latter data did not give exactly the expected advance in development, for, as we have more recently found, embryos in one litter may differ by from one to six somites. As it is evident then that carefully recorded breeding data may be misleading, the ages given to the stages in the plates must be understood to be only approximate, even though they are based on a considerable amount of accurately timed material. Nevertheless they indicate about what one may expect to find on those days.

A comparison of these data with those offered by Butcher (1929) in his excellent paper on the somites of the rat indicates agreement within one-fourth day or less, which is well within the range of variation. Our custom in determining age of embryos is to compare them with our standard series of photographs, of which the important ones are given here. We regret that space forbids publishing more, although it is hoped that means may be found for making them available to those who want them.

In view of the above, it has been the practice in recent years to place females (in the oestrous stage) with males in the evening, to examine them in the morning for the presence of spermatozoa, and to estimate ages as from the preceding midnight.

METHODS

For material younger than about twelve days, the uterus, after removal from an anesthetized pregnant female, and while being kept extended, was placed in Bouin's fluid until adequately fixed. It was then washed in a dilute solution (one-tenth of one per cent) of NaHCO_3 until white, thoroughly stained for from twenty-four to forty-eight hours in alum carmine, dehydrated, and prepared by dissection as described by Long (1936). Older embryos were removed from the foetal membranes while alive, and placed in Ringer's solution at room temperature, that being found more suitable than body temperature. By means of a small, two-way, brass stopcock with inlets attached to vertical glass tubes (or glass syringes) filled with Ringer's solution and Bouin's fluid to furnish the fluids under pressure, and with the outlet fitted with a fine canula inserted in the umbilical vein of the embryo, the vessels were first washed free of blood by allowing the heart to pump salt solution through them and out through an opening in the umbilical artery; then the embryo was fixed by gently forcing fixing-fluid into the vessels, and finally leaving it immersed in the fluid. Only in this way was it possible to preserve thoroughly the older embryos, with their nearly impervious skins, without shrinkage. This older

material was photographed while still in Bouin's fluid in order to avoid shrinkage that might be caused by alum carmine, the picric acid affording a good color for photographic purposes.

The technique of photographing has been described elsewhere (Long, 1936). For viewing the stereoscopic photographs see note on page 157.

When sections were desired, as in figures 4 to 10, the embryonic and part of the surrounding uterine structures were embedded, after being photographed, under a binocular microscope, so that they could be cut exactly in the desired direction.

Since the figures showing any uterine structures are oriented with mesometrial side toward the top of the plate, for the sake of brevity the mesometrial direction will be spoken of as up (above, higher); the antimesometrial as down (below, lower).

IMPLANTATION AND UTERINE STRUCTURES

No attempt is here made to consider at length the subjects of implantation and uterine structures which have been treated by Duval (1891) and Grosser (1909, 1927) for the rat, and by D'Erchia (1901), Burckhard (1901), Sobotta (1902), Asai (1914) and Grosser (1909, 1927) for the mouse. But figures 1 and 2 add to their accounts for the early stage of attachment of the blastocyst, and by plate 23 for midgestation membranes, decidua capsularis, and uterine lumen.

Several of our figures disclose the presence of a gelatinous, colorless, translucent, albuminous material, lacking blood corpuscles or cellular elements, labeled in some of the figures as "coagulated plasm." It regularly occurs in the lumen of the yolk sac, and often fills the fore- and hind-guts. In plate 23 it is shown typically as filling the uterine cavity, except for the space caused by shrinkage. In the specimen in figure 2 it was somewhat more transparent and tougher, completely filling the lumen except immediately above the blastocyst, and was removed with difficulty. Occasionally in the uterus it is apparently blood (fig. 3). Similar coagulable material occurs in both the extra-embryonic and the amniotic cavities, and usually has to be washed out if clean material is required for study and photographing.

Since it contains no haemoglobin, certainly not in cells, it cannot be spoken of as embryotrophy in Kolster's and Sobotta's sense. It would seem to be the same substance that is figured only by Parodi and Widakowich (1920) for the rat, and by Sobotta (1902, 1911) and Asai (1914) for the mouse as granular material in the cavity of the yolk sac.

It is assumed of course that the source of the material is the maternal blood stream, in the early stages at least, in contact with Reichert's membrane, through which it passes to bathe the (visceral) yolk sac as a nutrient fluid, and that some of it may pass, perhaps with alteration, on through the yolk sac and amnion.

In embryos removed in Ringer's solution the albuminous fluid which accounts for the "plasm" goes into solution or mixes with the salt solution and is

never seen. This fact undoubtedly accounts for its being overlooked by Everett (1935) in his excellent paper dealing with the function of the yolk sac. Whether there is any fatty substance in this "plasm" to account for the fat droplets found by Everett (p. 269) in the yolk sac epithelium, our material is not suitably preserved to show.

As Everett found in fresh material, so we too have observed in fixed, that Reichert's membrane is fairly tough. It is smooth and glistening. The amnion also is surprisingly strong.

ORIGIN OF EXTRAEMBRYONIC COELOM AND FOETAL MEMBRANES

The account may best begin with the stage shown in figures 4, 4a, and 4b, which is but slightly older than that in figures 31 and 32 of Huber (1915), in which the mesoderm has just started to form. The egg cylinder, which is approximately cylindrical, is cut open lengthwise a little to the left of the median plane, revealing the cavity extending its whole length. The cavity is slightly narrowed at about the middle where the amniotic folds are beginning to appear. The ectoderm lining the upper part, which is destined to be the cavity of the ectoplacenta, is clearly different from that lining the lower or amniotic portion, where the embryo takes its origin, as clearly shown by Huber. As a result of the presence of the primitive streak extending along the posterior side from just below the narrowest part of the constriction nearly to the lower end of the cylinder, the egg cylinder has a distinct bilateral symmetry the median plane of which is transverse to the uterus (see Widakowich, 1911). The axis of this embryonic portion of the cylinder is U-shaped and extends from the posterior end of the primitive streak down around the tip of the cylinder and up to its anterior end directly opposite the posterior end. Mesoderm growing out from the streak between the ectoderm and entoderm extends laterally as far as the dotted line in figure 4a, and slightly above (posterior to) the primitive streak, so that mesoderm does not at any place cross the median line. In other words, mesoderm is in two parts, right and left, as in the chick.

By inspection of figures 4a, and especially 4b, it will be seen that the anterior and posterior amniotic folds differ. The anterior is formed in part by the slight constriction of the wall of the cylinder, and in part by the thickening of the ectoderm as considered by Selenka (1884), and possibly by Robinson for the rat; whereas the posterior amniotic fold may be said to be formed chiefly as the result of the presence of mesoderm at the extreme posterior end of the primitive streak, which ends just below (anterior to) the middle of the fold. Lateral amniotic folds may also be considered to have begun to form as the result of a slight thickening of the mesoderm at its upper edge and to have extended about halfway around to the anterior median line.

It may be noted here that the anterior amniotic fold, as in the chick and other vertebrates, at first involves only the two primary germ layers, in an area which may properly be considered the proamnion. The latter seems to be

identified correctly by Robinson (1892) for the mouse (his fig. 15c), probably also correctly in the rat in his figure 16G, but overlooked in figures 14G and 14H.

In figures 4a and 5 to 10 the posterior fold is higher (i.e., nearer the mesometrial end of the cylinder) than the anterior.

In the next five stages illustrated (figs. 5-9) and in the first three of them particularly, the development or increase in the amniotic folds is accompanied by sharp folding or creasing in the entoderm, too distinct and definite to be artifacts. (See also figs. 6a, 7a, 9a.) This is true not only of the anterior and posterior but also of the lateral folds to some extent (fig. 6a).

Figures 5 to 7 show three further steps in the advance of the margin of the mesoderm as it grows from the primitive streak toward the median line on the anterior side, also as it extends higher above the primitive streak and lateral amniotic folds. Figure 5 differs little from figure 4, but in figure 6 the mesoderm, besides having advanced farther toward the median line, has extended above (posterior to) the primitive streak, where it constitutes a dense connection (labeled mesoderm) between right and left sides. Mesoderm has also formed a transverse connection between the two sides in the middle and upper part of the anterior amniotic fold; that is, mesoderm has invaded the proamnion as though to push back the fold in the entoderm and to take its place in the further growth of the amnion. As may be seen in figures 6a and 7a, the mesoderm is now thicker and continuous around the narrow central portion of the lumen of the egg cylinder. Together with the overlying ectoderm it forms the anterior, posterior, and lateral amniotic folds, which are destined to pinch off the upper ectoplacenta cavity from the lower amniotic cavity.

Within this mesoderm of the lateral amniotic folds there are meantime arising separate minute cavities (not shown in fig. 6a although present in the specimen) which in figure 7a are larger, as can be seen on the left side, and in the frontal section in 8a on both sides. Although not shown, these right and left cavities in both 7a and 8a are united in the posterior fold by minute irregular channels. These cavities enlarge still more (as in figs. 9 and 9a) as the right and left extraembryonic coeloms, which are still separate except for very small channels at their posterior ends. Very soon they are united by larger connections as in figures 10b and 10d. This description of the origin of the coelom is an agreement with the earlier accounts, especially those of Widakowich (1909) and Robinson (1892) for the rat, and of Sobotta (1911) for the mouse. In the earlier phases, as in figures 6a and 7, spaces, often considerable, appear between the germ layers as noted by Sobotta (1911) in the mouse.

With the establishment and expansion of the extraembryonic coelom (fig. 11a), the amnion and chorion (Sobotta, 1911) are formed and separated. They are connected for a time by a cord of ectoderm covered by a thin layer of mesoderm, whereas the coelom is lined by thick mesoderm (figs. 10b, 10d, 11a). This cord or connection was figured by Duval (1891), Robinson (1892), Cristiani (1892), and Widakowich (1909), and was called by Sobotta the

amnion navel cord (*Amniosnabelstrang*). It varies in position from central (fig. 10) to anterior (fig. 11), the latter being said to be characteristic of the mouse (Sobotta, 1911). Commonly it disappears early (fig. 12), but it may persist a considerable time (fig. 22, just anterior to the allantois). Since this connection corresponds exactly in position and method of formation to the seroamniotic connection in the chick, it may appropriately be renamed the chorioamniotic connection in the rat and the mouse.

The lateral walls of the extraembryonic coelom consisting of mesoderm and entoderm may now be called the yolk sac or the visceral portion of the yolk sac in which blood islands will soon be formed. Its mesoderm is continuous with both the mesoderm of the amnion and the mesoderm plate of the lower part of the egg cylinder along a line encircling the egg cylinder. As noted by Robinson (1892), the extraembryonic coelom becomes extended into the embryonic area by the splitting of the embryonic mesoderm into two layers (see figs. 13–22), thus extending the yolk sac and separating it from amnion and body wall.

It will be noted (fig. 12) that the allantois begins as the well-known solid outgrowth of mesoderm from the posterior end of the primitive streak, and that it extends rapidly into the exocoelom even before the chorion reaches the ectoplacenta.

EXTERNAL FORM

FIRST NEURAL FOLD TO SIXTEEN SOMITES (11 DAYS)

As has been seen, with the first appearance of the primitive streak just prior to the separation of the amniotic cavity from that of the ectoplacenta, the embryonic axis is established, and from the beginning is U-shaped. Since the embryo (including the primitive streak) arises on the sides and bottom of this deep cup-shaped cavity, it too is strongly bent with its dorsal surface concave—a fact which leads to a subsequent torsion to be described later.

The first part of the embryo to be formed (aside from the primitive streak) is the anterior portion of the neural folds, which appears as a slight V-shaped elevation in the ectoderm on the anterior side of the amniotic cavity (fig. 10c) at a time when the exocoelom is established as a single cavity (figs. 10b, 10d). The neural folds broaden and increase in height even before the head fold arises. (See also Adelmann, 1925.) The latter soon begins between the anterior end of the neural folds and the junction of amnion and yolk sac, and is well established before there are any somites and before the coelom has extended into the embryonic area proper (figs. 14, 15). Figure 14 clearly shows the U form of the embryo, with the dorsal sides of the anterior neural folds facing the dorsal side of the primitive streak.

From the beginning, the inner surfaces of the neural folds are strongly convex (fig. 15) and their margins are rolled laterally and under, while the bottom of the neural groove is sharp and deep. With the appearance of the head fold the anterior edges of the neural folds also are rolled under, and as the head fold becomes more prominent, the anterior end of the developing

brain bends ventrally and comes into contact with the ectoderm covering the heart area (plates 14–17). This outward rolling continues up to the time of closure of the neural tube (fig. 21*a*). (Compare Adelmann, 1925, pl. 1.)

The first grooves of segmentation of the neural tube (Adelmann 1925) are clearly evident in these early stages (fig. 15), and subdivision continues while the neural groove is wide open (figs. 18*b*, 21, 22*b*).

Somewhat surprising is the amount of irregularity and asymmetry in the neural folds of the early stages (fig. 15), and in the beginning of lateral bending sometimes found in stages of 2 to 3 somites (not included in the plates). Moreover, the correlation of the development of somites and other parts is not constant within small limits (e.g., compare the embryos of figs. 18 and 21).

The primitive groove broadens and deepens and is continuous with the neural groove (figs. 16*b*, 18*b*, 21*a*).

The visibility and clearness of the somites differs greatly in different embryos, so much so that often the number can be determined only by sectioning. It may also be noted here that in later stages the size and shape of somites, and their distinctness one from another, is subject to great variation. In accordance with Butcher (1929), we consider the second somite as the most anterior one having a distinct anterior boundary.

As in other vertebrate embryos, the anterior intestinal portal appears with the establishment of the head fold and the primitive fore-gut (fig. 14). In the earliest stage it is well forward of the first somite, but later it comes to lie farther and farther posteriorly and at levels of more posterior somites (see also Butcher, 1929, pp. 387–388). Although we are aware of the difficulties of explaining this movement, we offer in figure 17 an embryo in which the margin of the portal in the median line is a sharp, deep notch. This might be taken to indicate a process of concrescence of the lateral margins of the portal in the median line, or only a peculiar variation of no significance.

At the 6–7-somite stage the posterior intestinal portal first becomes evident, and at the same time the tail fold and the extreme posterior parts of the lateral body folds make their appearance (fig. 18*b*). The posterior portal moves cephalad, but there is no evidence to show whether by concrescence or otherwise. The mid-gut at first is wide open and not even a groove, being the convex outer surface between the two portals.

The first beginnings of the optic vesicles are the optic fovea which appear on the inner surfaces of the neural folds at their anterior ends (figs. 18, 18*a*, 21*b*). They can be followed as deepening pits until the neural tube closes, when they appear as lateral enlargements of the anterior end of the head (fig. 22, etc).

Closure of the neural tube begins at about the 6-somite stage (fig. 21*a*), in the region of the second and third somites, proceeding rapidly caudad past the last somite (fig. 22*b*). It next occurs in the diencephalon (figs. 22, 23, 23*a*). There are thus left open the anterior and posterior neuropores, the midbrain, and the anterior part of the hindbrain. Final closure is accomplished in the order of midbrain, anterior neuropore, hindbrain (which closes from both

ends), and lastly the posterior neuropore, which remains open until about the 21-somite stage.

Although in sections the precardiac mesoderm may be detected as early as figure 12 and the pericardial cavities may be seen just uniting at a stage between figures 15 and 16, the forming cardiac folds cannot easily be identified until about the stage of figure 16. From then on, the heart grows with great rapidity during the 5-7-somite stage (figs. 17-21) until at 11 somites (fig. 23a) it is a prominent protrusion under the forebrain, covered by the thin body wall.

Even before the cardiac loop is well formed close under the forebrain, the dorsal ends of the mandibular arches are first evident as slight swellings (fig. 21), which become more and more prominent as they elongate ventrally. By the 8-9-somite stage (fig. 22) they are distinct enlargements pressed closely both against the brain under the optic prominences on their anterior side and against the cardiac swellings on their posterior side, as though pushing in between the two but not meeting each other ventrally. At this same time a slight swelling behind the mandibular arch may be seen as the forerunner of the hyoid arch. This may be observed in figure 23a, and later, in figures 24 to 26, as a distinct arch between the first two visceral furrows, its ventral end merging with the body wall over the heart. Although the mandibular arches have not met at their extreme ventral ends (as though kept apart by the bulbus of the heart), the oral cavity is present as a mere crevice between the under side of the head (forebrain) and the area between the arches (fig. 25) at a stage in which the oral plate has already been formed and ruptured.

The fundament of the inner ear may first be observed at 10 somites (fig. 23) as a slight depression just above where the second furrow soon is to appear. It deepens as the furrow becomes deeper (fig. 24) until by 16-17 somites (fig. 25) it is a distinct pit. With increase in depth the opening of the pit narrows, as at 21 somites (fig. 27a), and finally closes at 26 somites (fig. 28).

There has been going on during the period of the developments just described a striking series of changes by which the embryo becomes constricted from the yolk sac and rolls over so that the curvature of the dorsal surface becomes convex and the body takes on a partly spiral form. These changes were first noted for the mouse by Ravn (1894) and described for the rat by Widakowich (1909), whose account agrees essentially with the following brief description, except that Widakowich speaks of the posterior end of the embryo as turning to the left (p. 291) while his figure shows both anterior and posterior portions turning to the right.

The constriction from the yolk sac is the result of the extension of the head and tail folds (figs. 21b, 22a) and the appearance of the lateral body folds (figs. 23, 23a) for the most part; but the transition from the wide-open, flattened mid-gut to a narrow crevicelike furrow is dependent also on the change in curvature of the whole body, after which change separation from the yolk sac is rapid and complete (cf. figs. 23-25). Even after the separation is accomplished there is still a slight groove in the yolk sac marking the former line of attachment of the stalk (figs. 28, 42).

Since the embryonic coelom arises as an extension of the original extra-embryonic body cavity, the two are from the beginning always in continuity, the former being wide open into the latter. With the development of the lateral body folds they become constricted one from the other until they are entirely separated at twelve and one-half to thirteen days (figs. 29–30), a time when by the approximation of the umbilical and vitelline vessels the umbilical cord may be said to begin.

The slight asymmetry already noted in the early stages can be considered as the beginning of the change in form. It is clearly evident at 6 somites (fig. 21), where both the anterior and posterior ends are beginning to turn toward the right as a result of simple bends in the longitudinal axis. This also may be seen in figures 18 and 16, but it is much less marked. The bending is soon accompanied by twisting of the body of the embryo at both ends whereby the latter begin to turn over on the left side. Indeed, since the embryo lies in the bottom of a deep cuplike cavity, bending of the axis amounts substantially to turning over onto the left of the anterior and posterior portions (figs. 22, 23). This bending and twisting, contrary to what might be expected, is more advanced in the tail than in the head (fig. 22) in correlation with the development of the allantois, a point overlooked by Widakowich. For it will be seen that the allantois has not yet reached the ectoplacenta when the torsion first begins (figs. 18, 21) in the head, but has established a broad connection before torsion in the posterior part is well advanced (fig. 22). As will presently be seen, this state of the allantois is also correlated with the coiling of the tail to the right of the head. It will easily be understood that when the torsion of the two ends, which amounts to rolling onto the left side, has reached the middle of the body (cf. figs. 23–25), the whole body comes to lie on its left side, with the result, since it is confined within the amniotic cavity, that it is strongly bent in a direction opposite to the original curvature. This is accomplished during the 13–15-somite stage, and is finished by 16–17 (fig. 25).

It will be noticed that the point of attachment of the allantois to the ectoplacenta is opposite the yolk stalk, and that since the allantois does not elongate rapidly, the tail end is drawn away from the yolk sac toward the right of the embryo as the body rolls on its left side, as can be seen clearly in figures 23 to 25, and later in figures 43 and 44 (pl. 13).

It still remains to be noted that while the yolk stalk is intact (figs. 23–25), the rolling over of the embryo has strongly drawn the stalk to the left side, bringing about considerable internal asymmetry.

Since this stage of 16–17 somites is distinct, and easily identified in development, it may be well to note the chief external features in addition to the coiling (fig. 25, etc.). The neural tube is closed, except for the posterior neuropore near the tip of the considerably elongated caudal portion from which the allantois arises. The lateral swellings of the optic vesicles, the first two arches, heart, and midbrain are conspicuous, and the auditory pit is fairly deep and wide open. The oral pit or cavity is indicated externally only by the cleft between the mandibular arch and head. The maxillary processes, cerebral enlargements, and limb buds have not yet appeared.

MORE THAN SIXTEEN SOMITES (11 DAYS TO 19½ DAYS)

The posterior neuropore, still open at 16 somites, is closed at 21 (fig. 27), and the auditory pit with a minute porelike opening at 21 is entirely closed and cut off from the ectoderm at 24 somites.

The cerebral hemispheres scarcely existent at 16 somites (fig. 26) are clearly evident at 21 (fig. 27) as enlargements in front of the optic swellings, as though pushing the vesicles back to a point just in front of where the maxillary processes are soon to appear. By the time the latter are beginning to appear, the lens vesicle is a shallow pit (21 somites, fig. 27) which rapidly deepens to be cut off at 34 somites (fig. 29). From then on the eye is outlined externally by the developing eyelids, as at 14 to 15 days (figs. 32, 34). Mean-time, the cerebral hemispheres become more and more prominent as enlargements above the developing face and snout.

Early in this second period the yolk stalk diminishes (21 and 24 somites), until at 26 somites (fig. 28) the intestine is entirely separated from the yolk sac. During this time the body wall in the posterior part of the body is becoming continuous ventrally to cut off the coelom within the embryo from that without.

Externally the only visceral arch to be added is the third, together with the third furrow. They come to be depressed and partly covered by the second arch at 34 somites (fig. 29), the second visceral groove marking the outer end of the cervical sinus (figs. 30-32a).

The maxillary process first is distinguishable at 21 somites (fig. 27) and the olfactory pit at 26 to 30 somites (fig. 28a), the former as a slight elevation at the upper end of the mandibular arch, continuous with it and extending toward the eye; the pit as a depression on the ventrolateral aspect of the prominences of the cerebral hemispheres. By 34 somites (fig. 29) both are well developed. The olfactory pits have become deeper and vesicular. The caudal ventral ends of their narrow, external openings are bounded by the ends of the maxillary processes (fig. 29a) which come into contact with what may now be called the medial and lateral nasal processes, clearly seen in lateral and ventral views. The eye lies at the upper end of the groove between the maxillary and lateral nasal processes. While the fusion between the maxillary processes and the nasal processes, and between the two median nasal processes, is becoming complete to form the upper jaw (13½ days), the lower ends of the mandibular arches, now only separated from each other by a deep cleft at 26 somites (fig. 28a), are fusing to constitute the lower jaw (fig. 29a); the oral cavity or pit being the well-defined depression bounded by these structures.

It will be noted, in comparing the 12½- and 13½-day stages that the external nares or nostrils are smaller and narrower, and the olfactory vesicles are not only relatively but also actually closer together and are separated from the oral cavity by thin membranes which mark the positions of the primary internal choanae. The continuation of these processes is easily followed in the succeeding figures, which also show the developing vibrissae on the lateral

swellings or beginnings of the cheeks. Attention may be directed here to the other groups of tactile hairs on the head and of hair follicles over the body.

As the third visceral arch and furrow become submerged, the ventral end of the first furrow becomes deeper to form the external auditory meatus, while from the margins of the remainder of the furrow the external ear itself is derived.

Throughout the earlier period and up to thirteen and one-half days the ventral body wall in the thoracic region was so thin that much of the heart was visible, but it now becomes thicker. Mammae appear as early as fourteen and one-half days.

The first clear sign of the anterior limb bud can be seen at 21 somites (fig. 27), between about the sixth and tenth somites, as a lateral swelling. At about 34 somites (fig. 29) the posterior limb is similar to this, whereas the anterior is longer and paddlelike. The anterior continues to be more advanced than the posterior. Both become longer, and the distal ends broaden, flatten, become five-angled, and then lobed. The lobes become both separated by deeper and deeper clefts and transformed into digits with claws, ventral foot pads meanwhile appearing. Whereas the anterior foot begins with five digits, the first, always a little smaller, becomes reduced and never acquires a claw, until by nineteen and one-half days it is reduced to a mere knob on the inner margin of the foot. The fifth digit is smaller than the other three. On the hind foot the middle three toes come to be of equal length, the other two being shorter.

At thirteen and one-half days the head, when clearly distinguishable from the trunk, is nearly as large as the rest of the body; but from then on the proportions steadily change until at nineteen and one-half days the body posterior to the neck has approximately three times the volume of the head.

This change in proportion begins about the time when the umbilical cord is first indicated and the intestine forms a simple loop which pushes ventrally as the beginning of the umbilical hernia. The latter can just be detected at thirteen and one-half days, is seen better at fourteen, and at fourteen and one-half and fifteen appears clearly as coils of intestine covered by a thin membrane. By seventeen days (fig. 37) the hernia is a large, balloonlike, spherical mass pressed against the body and united to it by a narrow connection. From the side of the hernia opposite the body emerge the two umbilical and the two vitelline vessels, the former passing to the placenta and the other two in the opposite direction to the yolk sac. Beginning at seventeen and one-half days the hernia rapidly is reduced in size by the withdrawal into the peritoneal cavity of the coils of intestine, so that by eighteen days scarcely any is left. These changes are accompanied by the closure of both the eyes and ears until the eyelids are in contact with each other and the external ear is fastened like a flap over the auditory canal. The rapidity of these processes is shown by the two 17½-day specimens from the same litter (figs. 38, 39). The reduction of the hernia leaves a short umbilical cord, connected directly to the ventral body wall.

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EXPLANATION OF PLATES

THESE STEREOSCOPIC PHOTOGRAPHS were purposely made with even lumination (see Long, 1936) and therefore without deep shadows, in order to show as much detail as possible. Consequently, in order to appreciate features otherwise invisible it is important to obtain the third-dimensional vision in the illustrations. This may easily be done without a stereoscope, as follows. Place a piece of cardboard (12 in. long by 3 to 6 in. wide) on end between the photographs of a pair and with the nose in contact with the upper end, and the pictures *equally lighted*, and look at them as though at a distant object until the two images merge. If adjacent photographs are distracting, they may be covered. With a little practice one may dispense with the card and pass from one to another of the figures on a plate while viewing them stereoscopically and study them in detail.

For use with a stereoscope it is best to cut up the plates and mount the members of a pair on a card, $2\frac{3}{4}$ to 3 inches apart from center to center.

Figures bearing the same number are from the same embryo.

Yolk sac refers to the visceral portion of the entire membrane.

Reichert's membrane includes the parietal portion of the yolk sac entoderm, where it is used on the photographs.

The figures are not completely labeled, in order to avoid crowding, it being left to the reader to identify many structures by comparison of figures.

PLATE 11

Explanation of figures

1. 8½ days. Transverse dissection through uterus.
- 1a. Enlargement of lumen of uterus and blastocyst.
2. From same litter as figure 1. Uterus dissected sagittally. A colorless and translucent coagulum which filled lumen of uterus has been removed. Lumen unobstructed. Note thickenings on either side of blastocyst which in figure 3 cause complete obstruction of lumen.
3. 8¾ days. Sagittal dissection through uterus. Lumen filled with coagulated blood.
4. 8¾ days. Transverse dissection.
- 4a. Blastocyst cut open sagittally slightly to left of median plane.
- 4b. Cross section through amniotic folds, accurately drawn except cells.

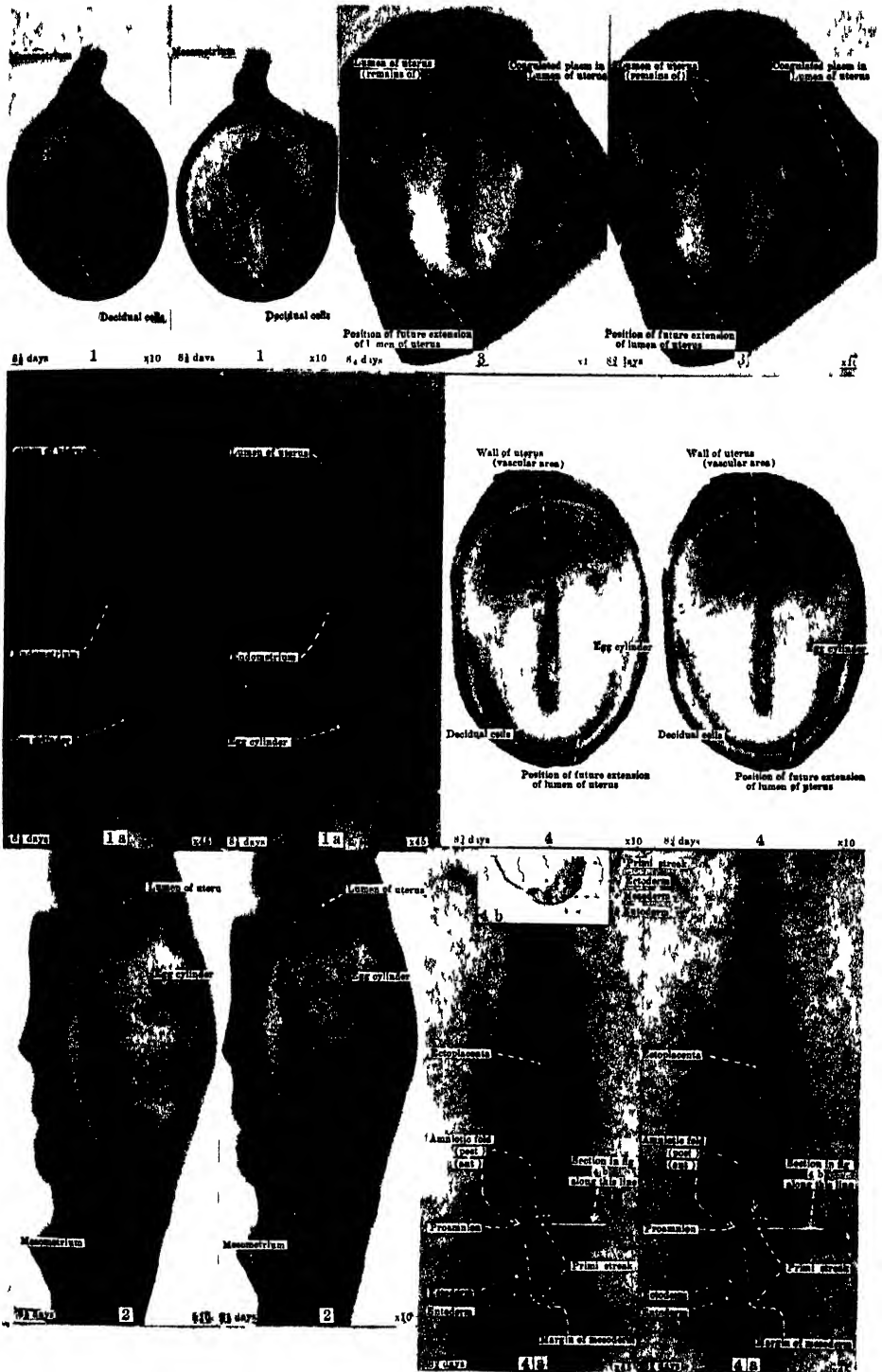


PLATE 12

Explanation of figures

All are figures of sagittal dissections through the egg cylinders. All specimens were actually sectioned after careful orientation. Plane of sectioning and position of section drawn in figures labeled "a" indicated by solid white lines.

5. 8 $\frac{1}{4}$ days. A little to left of median plane and primitive streak; therefore shows all three germ layers in posterior half. Dotted line shows extent of mesoderm.
- 5a. Through upper end of primitive streak.
6. 8 $\frac{1}{4}$ days. Mesoderm continuous from right to left in small area bounded by dotted line in and above anterior amniotic fold. No coelom except a few isolated crevices not shown.
- 6a. Cross section two sections above end of primitive streak.
7. 9 days. From same litter as figure 10. Mesoderm has reached its greatest extent.
- 7a. Through extreme upper end of primitive streak.
8. 9 days. Embryos of figures 8, 9, and 11 from same litter. Mesoderm, amniotic folds, and coelomic spaces about the same as in figure 7. Deep amniotic folds in entoderm.
- 8a. Frontal section showing lateral amniotic folds and their incipient coelomic cavities.
9. 9 days. Large right and left coeloms, entirely separate except for small connecting channels at posterior end, not shown.
- 9a. Transverse section just above primitive streak. Shows connection between ectoplacenta and amniotic cavities greatly reduced with enlargement of coeloms.

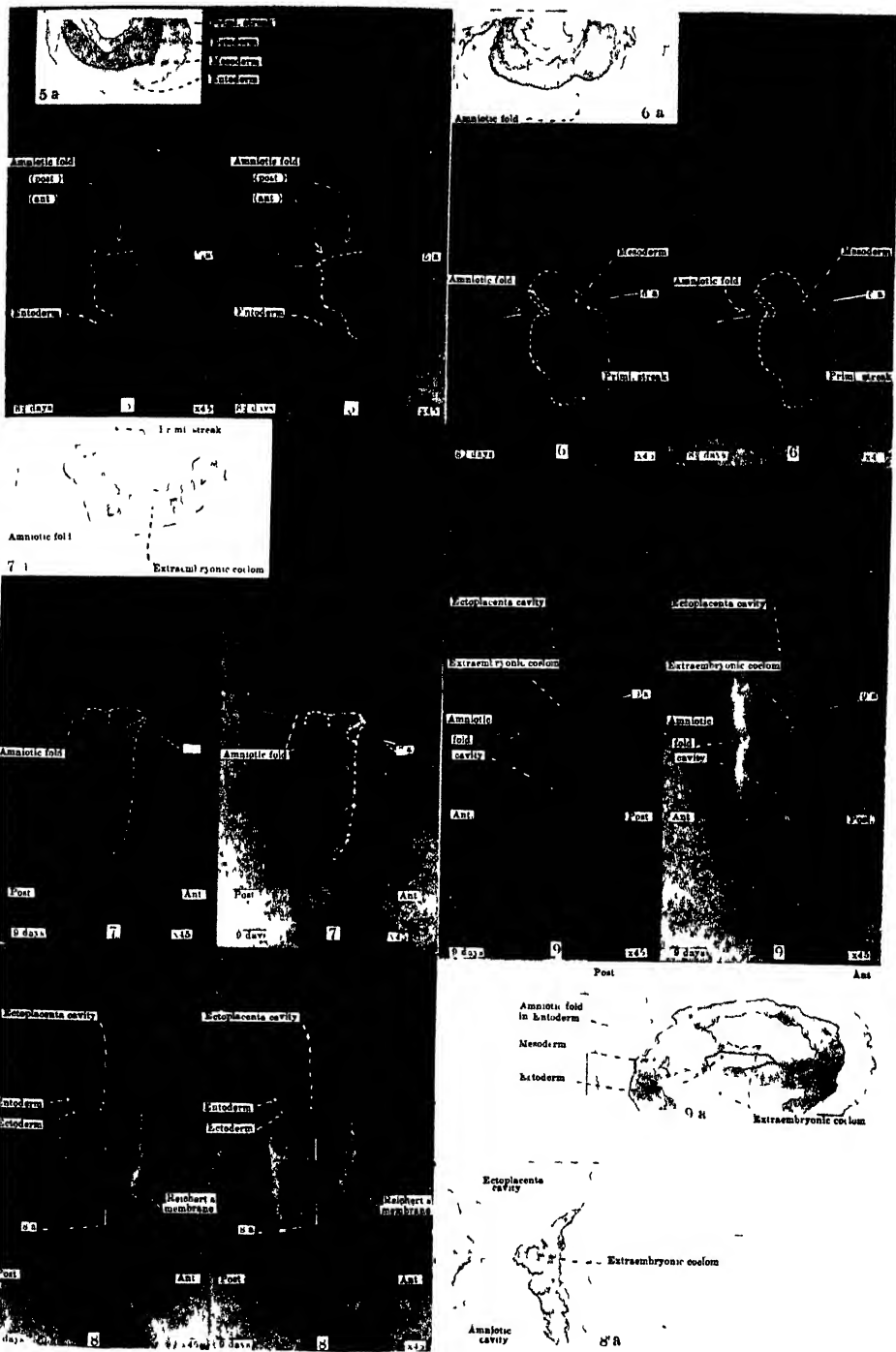


PLATE 13

Explanation of figures

10. 9 days. Same litter as in figure 7. The egg cylinder after being cut open parasagittally to the right of the median plane, was cut across just above the amniotic folds, and then photographed as in *a* and *b*. It was then again transected just below the amniotic folds, the lower portion with the amniotic cavity being laid open as in *10c*, while the coelomic portion was sectioned transversely, *10d* being about through the middle of the coelom. *10d* shows the formation of the chorioamniotic connection by the joining of the right and left extraembryonic coeloms like those of figure *9a*. The neural folds are very inconspicuous elevations on the anterior side of the amniotic cavity in *10c*.
11. 9 days. From same litter as embryos of figures 8 and 9. Transverse dissection of uterus.
- 11*a*. Egg cylinder dissected sagittally. Chorioamniotic connection far anterior. Left neural fold cut obliquely. No allantois.
12. 9 days. Coelom larger. Chorioamniotic connection very slender, torn loose from chorion. Allantois started.

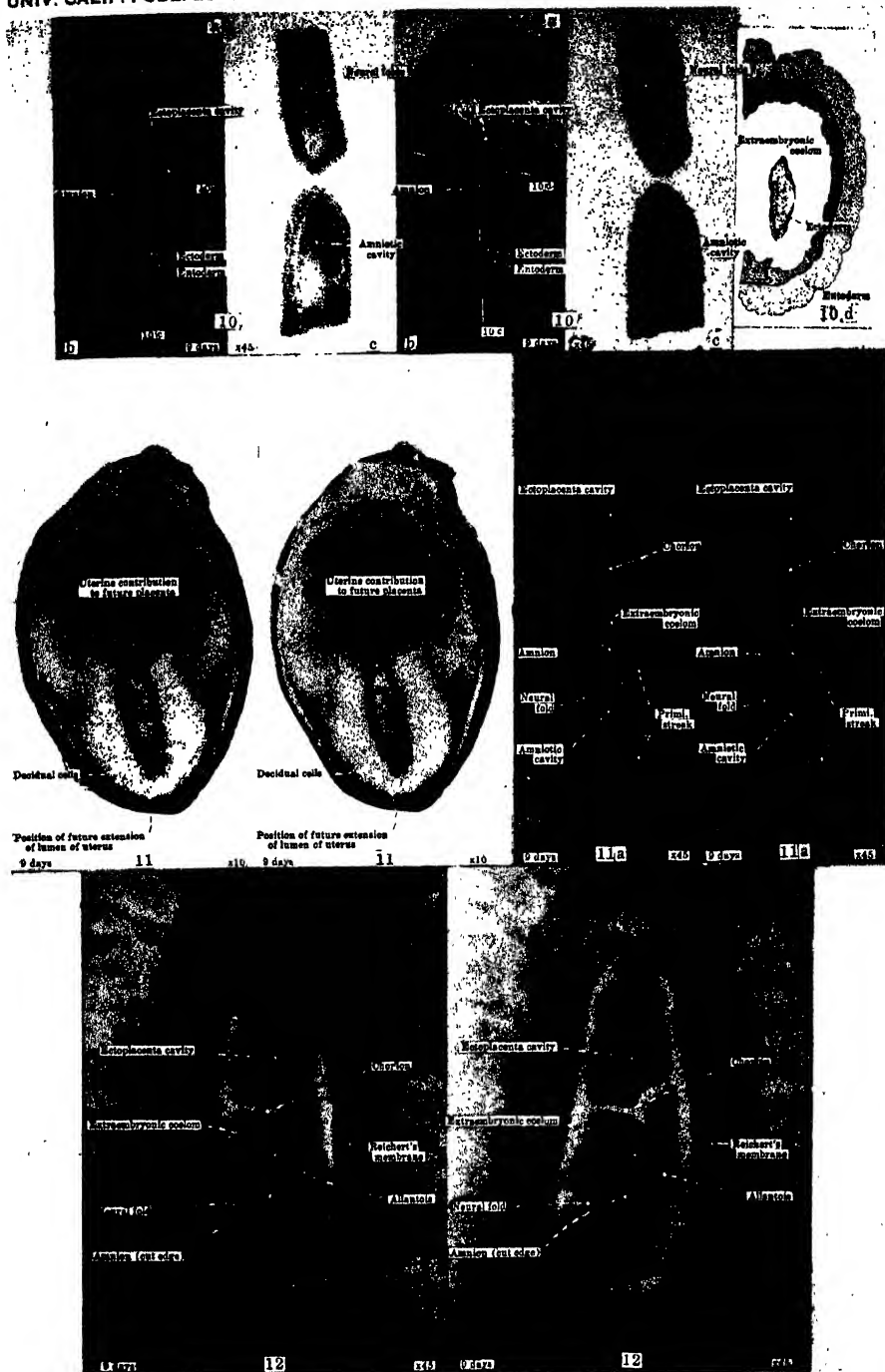


PLATE 14

Explanation of figures

13. 9¼ days. Dissected from left.
- 13a. Dorsal view of embryonic area. The coelom has not yet invaded the embryonic area.
14. 9½ days. Median sagittal dissection. Blood-filled spaces next to Reichert's membrane. Only part of the coagulated "plasm" left in the yolk sac.
15. 10 days. No somites. Dorsal view of embryo.



PLATE 15

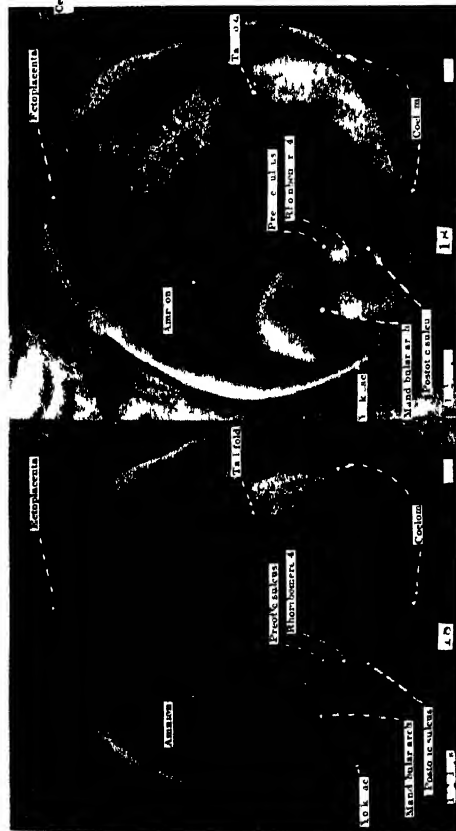
Explanation of figures

- 16. 10¼ days. 5 somites. Same embryo as in figure 20. Some of albuminous material left in yolk sac.
- 16a. Ventral view.
- 16b. Dorsal. Slight curvature to right.
- 17. 10¼ days. 4–5 somites. To show what appears like confluence in margin of portal.

PLATE 16

Explanation of figures

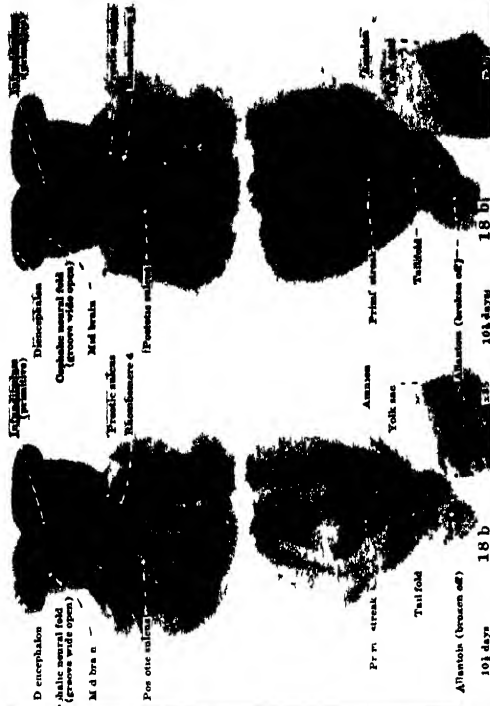
18. 10½ days. 7 somites. Extraembryonic coelom has extended into the embryo. Allantois has reached and touches ectoplacenta.
- 18a. Ventral. Earliest evidence of optic fovea. Splanchnopleure (yolk sac) covering the heart has been removed.
- 18b. Dorsal.
19. 10 days. First somites marked out by cell arrangement but not separated. Uterus dissected longitudinally. Shows expansion and extension of uterus lumen about mass of decidual cells.
20. 10¼ days. 5 somites. Same embryo as in figure 16. Dissected longitudinally, also cut crosswise, as in figure 19.



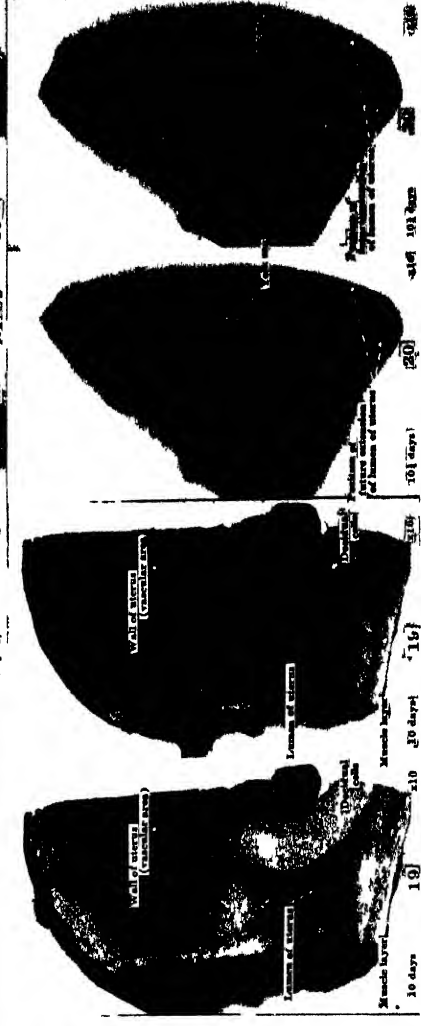
Optic fovea



Optic fovea



Optic fovea



Optic fovea

PLATE 17

Explanation of figures

21. 10½ days. 6 somites. Dissected from right far enough to open pericardial cavity. Allantois still free. Most of amnion removed.
- 21*a*. Dorsal.
- 21*b*. Ventral. Right optic fovea cut across. Notice that while allantois is less advanced than in the embryo of 7 somites (fig. 18), the nervous structures are further developed. Decided curvature to right.
22. 10¾ days. 9 somites. Allantois fused to ectoplacenta. Slender, exceptionally late remains of chorioamniotic connection.
- 22*a*. Ventral. *b*., dorsal, *c*., anterior. Decided rolling to left at posterior end correlated with attachment of allantois to ectoplacenta. Rolling of head beginning (*c*)



PLATE 18

Explanation of figures

- 23. 23a. 10 $\frac{3}{4}$ days. 11 somites. Both anterior and posterior parts of body rolled over 90 degrees. Still broadly attached to yolk sac.
- 24. 10 $\frac{3}{4}$ days. 14 somites. Almost completely rolled over. Broad attachment to yolk sac.
- 25. 11 days. 16 somites. Rolling over and coiling complete. Tail position to right of head correlated with attachment of allantois. Yolk sac nearly separated from yolk stalk.
- 26. 11 days. 16 somites. Face view. Mandibular arches still widely separated ventrally.

In the following plates, except plate 13, the embryos are shown with the amnion removed up to the body wall.

PLATE 19

Explanation of figures

- 27 and 27a. 11¼ days. 21 somites. Yolk stalk slender with restricted connection to yolk sac, of which only a small piece is shown. Extensive connection between embryonic and extraembryonic coeloms (fig. 27a) along line of attachment of amnion. Mandibular arches in contact at ventral ends (fig. 27b). Maxillary process first appears. Auditory vesicle nearly closed (27a). 3 visceral arches visible.
28. 11½ days. 26 somites. Final separation from yolk sac, leaving vitelline vessels. The label "yolk stalk" represents former place of attachment to yolk sac. Posterior limb buds are slight swelling, not shown in figure.
- 28a. Face. First evidence of olfactory pits.
29. 12½ days. 34 somites. Allantois and vitelline vessels still widely separated. Lens vesicle nearly closed. Auditory vesicle completely covered by ectoderm. Hyoid arch commencing to cover third. Beginning of cervical sinus.
- 29a. Face. Mandibular arches beginning to fuse.

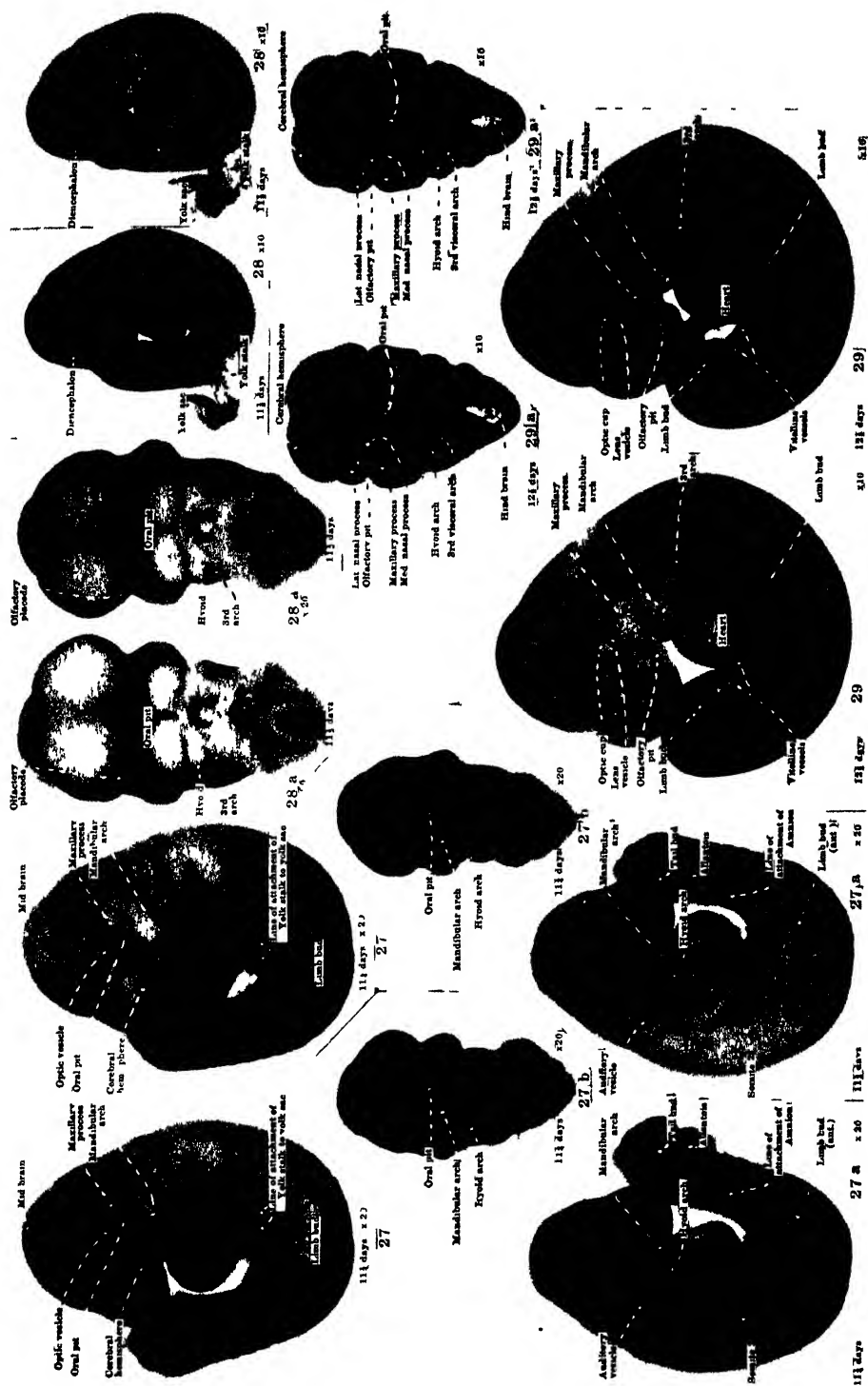


PLATE 20

Explanation of figures

- 30. 13 days. With lengthening of tail and closure of coelom the umbilical and vitelline vessels are brought closer together as the beginning of the umbilical cord.
- 30a. Face. Beginnings of upper and lower jaws.
- 31. 13½ days. Beginning of hernia. No digits. Feet paddlelike.
- 31a. Face. Median nasal processes fusing, and primary internal choanae about to be formed. Some evidence of palatal processes.
- 32. 14 days. Fundaments of feet angular.
- 32a. Face. Beginnings of tongue and palatal processes. Mouth closing. Vibrissae follicles visible.
- 33. 15 days. Face. Mouth nearly closed.

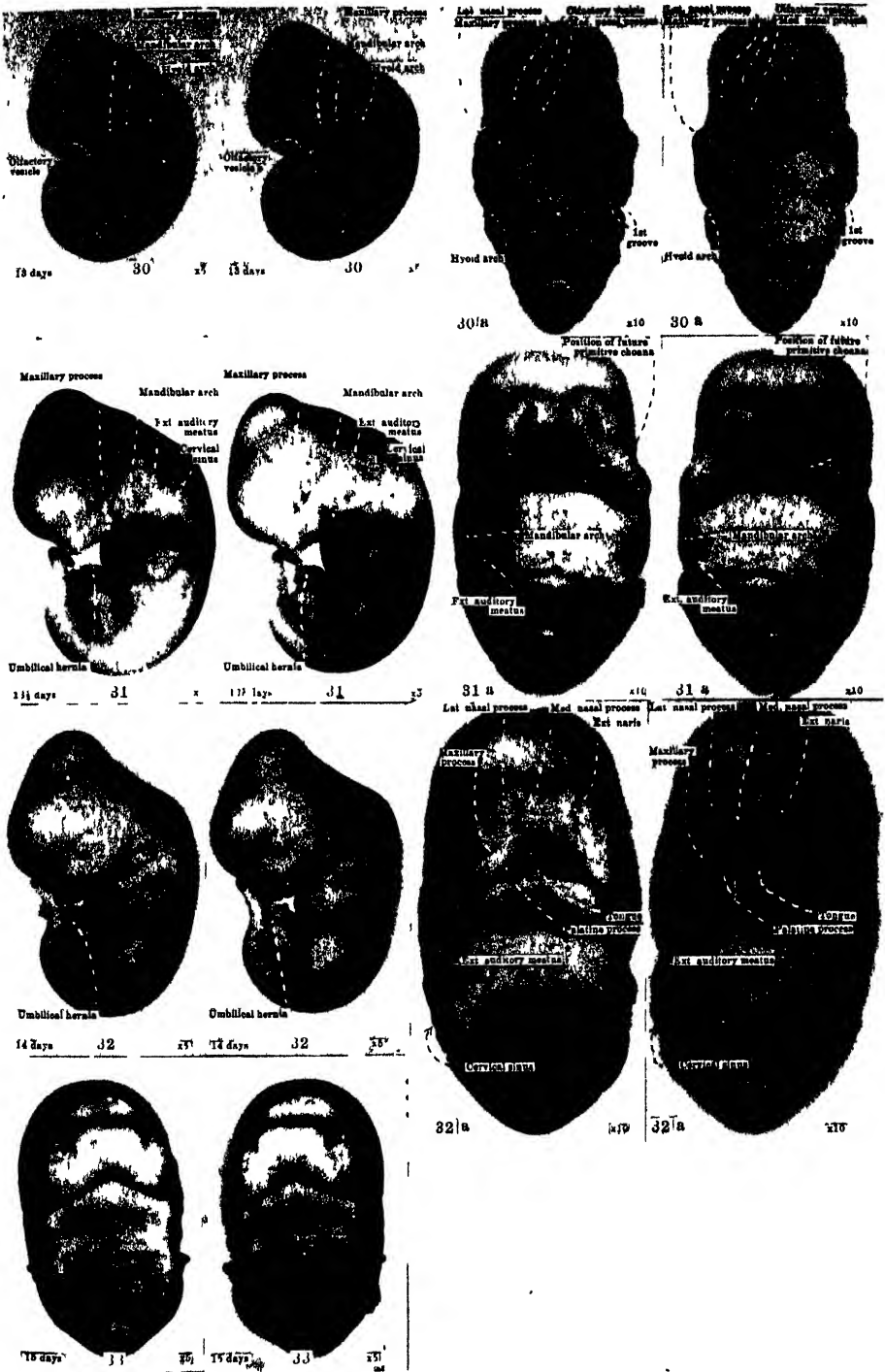


PLATE 21

Explanation of figures

34 to 36*a*, illustrate increase to maximum of hernia, advance in face, opening of mouth with enlargement of tongue, and closure with withdrawal of latter. Eyes and ears open. Follicles appear over surface of body. Mammae. 36*b*, ventral surfaces of limbs, anterior above, posterior below.

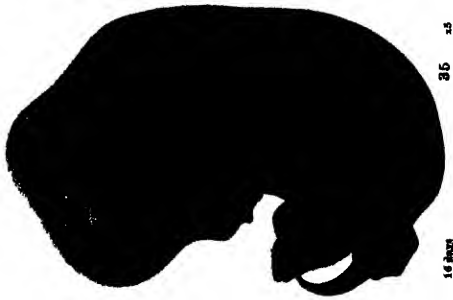
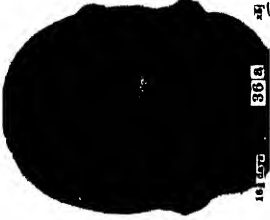


PLATE 22

Explanation of figures

Feet as in plate 21. Withdrawal of hernia, accompanied by closure of eyes and ears. 38 and 39 from same litter. Neck more distinct.

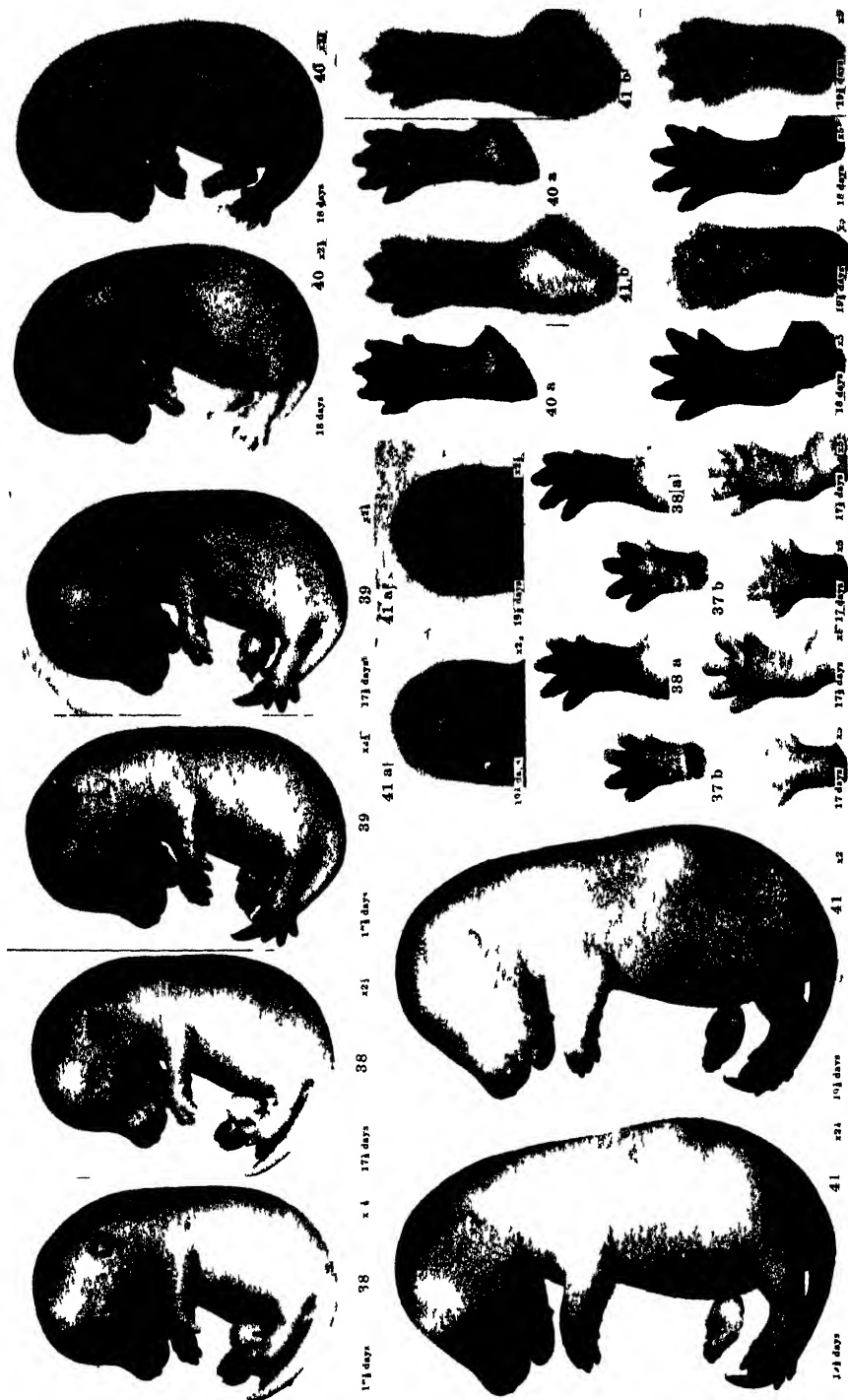
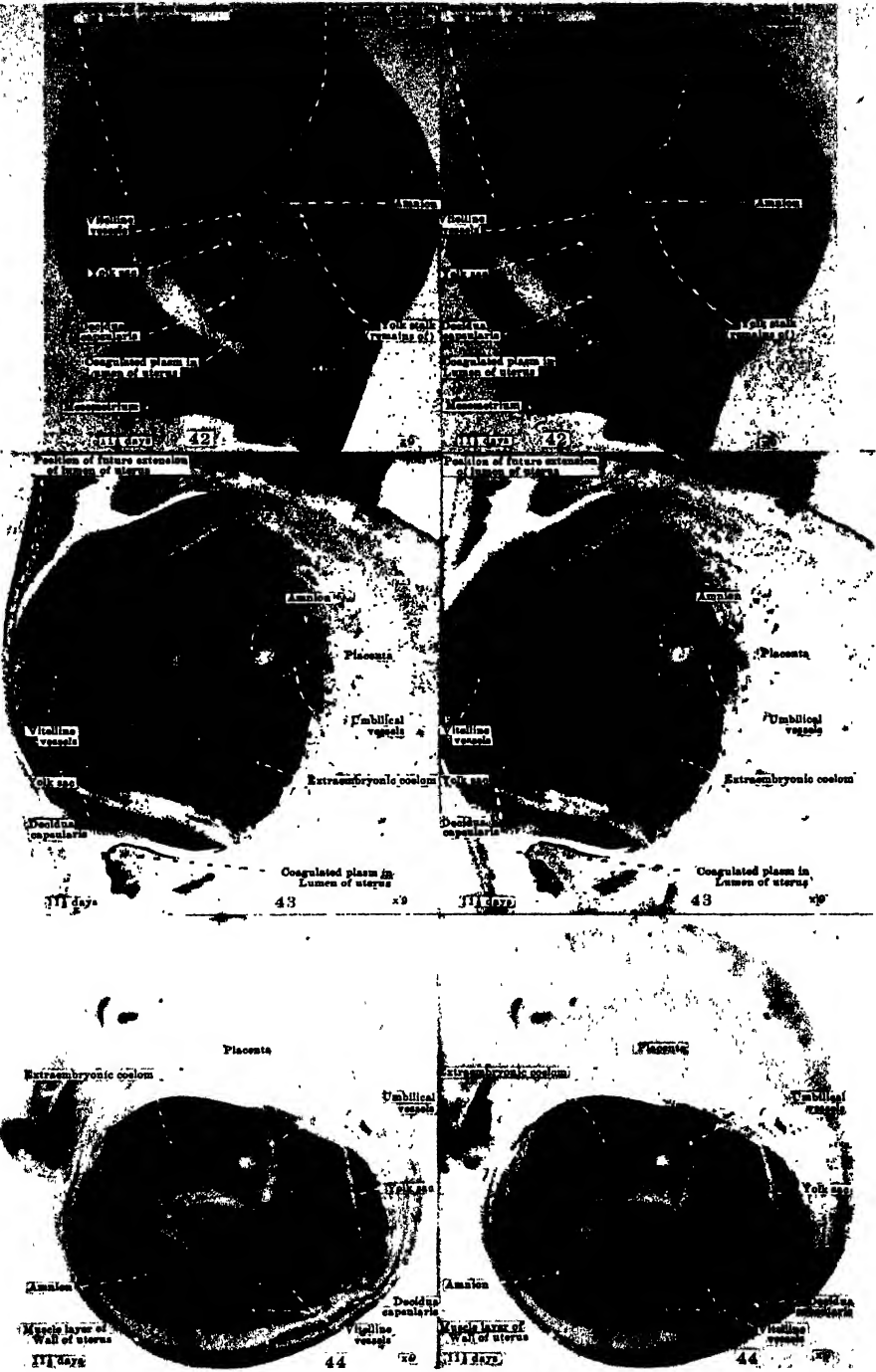


PLATE 23

Explanation of figures

42, frontal; 43, sagittal; 44, transverse dissections of uterus about eleventh day of pregnancy, to show membranes and relation of embryo to them. Uterine lumen reëstablished except for a narrow band extending from forming placenta laterally and antimesometrially. Lumens of uterus and yolk sac filled with colorless, plasmlike coagulum, which in a flocculent form occurs also in the extraembryonic coelom in figure 42. Notice constancy of orientation of embryo in uterus in spite of slight collapse of capsularis and yolk sac in 43; also apparent suspension of embryo between vitelline and umbilical vessels; very large exocoelom. 43 and 44 show relation of amnion to tail, body wall and umbilical vessels. Compare with figures 23, 23*a*, 24, 27.



**FURTHER STUDIES
IN REGULATORY DEVELOPMENT
OF TRITURUS TOROSUS**

BY

RICHARD M. EAKIN

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BY

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INTRODUCTION

THE NATURAL conditions which a living organism encounters in any environment are characteristically inconstant. Pressure, temperature, radiations, and the relative concentrations of various elements and compounds are only some of the many variable factors of the external environment. Conditions within the living organism, however, are relatively stable, as is required for normal development and proper physiological activity. It follows, therefore, that those adverse factors of the external environment which are continually besetting the course of development and the processes of physiological activity are overcome somehow by regulative responses, enabling the organism to preserve and maintain unity of form and harmony of activity.

The phenomenon of regulation may be observed in forms of life from the smallest microorganism to the most highly developed and most delicately balanced living system. Fastness of bacteria to progressively higher concentrations of phenol, and tolerance of protozoa to increasing quantities of trypan red are examples of regulative activity, as is the homeostasis of neutrality in the blood of man. Organisms vary, however, in the degree of regulation which is essential to maintain a uniform and integrated system of developmental and physiological processes.

It may be convenient for purposes of consideration to distinguish two aspects of regulation: first, the maintenance of function, and, second, the maintenance of form.

THE MAINTENANCE OF FUNCTION

The regulation of physiological activities in the so-called prefunctional period of development is difficult to demonstrate. Although certain functions, such as muscular contraction or nervous conduction, have not appeared as yet, the basic physiological processes, such as metabolism, respiration, and excretion, are nevertheless even then taking place. Since harmony of activity is essential for continued existence, we conclude that regulation of physiological processes occurs in the later part of the prefunctional, as well as in the functional, period of development.

When an organism enters the second period of development, however, activities become more intense and more diversified, and their regulation and integration more evident. In higher forms the maintenance of bodily function is accomplished largely by the nervous and endocrine systems. By experimentally controlling the activity of these two systems, at least in part, one may

analyze the regulation of certain physiological activities. Professor Walter B. Cannon presents in his book *The Wisdom of the Body* a clear picture of the regulative activities involved in the maintenance of many homeostatic conditions, such as the relative constancy of bodily temperature and of blood-sugar concentration.

THE MAINTENANCE OF FORM

During the periods of development when primary organic form is being established, regulatory processes are operating constantly to insure unity and maintain harmony of form. There may be loss of materials through injury; there may be an addition of materials through fusion with another embryo; formative movements may be disturbed by mechanical obstructions; or the rate of development may be too greatly accelerated in one part of the embryo, or excessively inhibited in another. Regulatory processes, however, serve to circumvent these disorganizing forces and to maintain the structural integrity of the organism.

METHODS OF EXPERIMENTAL ANALYSIS

Attempts have been made by a number of investigators to analyze experimentally the problem of regulation by subjecting organisms to adverse forces or conditions during the period of determination of various organ anlagen. Two general methods of study have been employed: (1) the removal of materials, and (2) the addition of materials. As examples of the former may be cited the isolation of egg fragments, the separation of early blastomeres by any of the various specific methods, the constriction of blastulae or gastrulae, the extirpation of certain materials, and the killing of particular portions of the embryo. The second general method may be illustrated by the fusion of eggs or early blastomeres, the implantation of certain materials, and the fusion of lateral portions of gastrulae in such a manner that the composite embryo thus formed has an excess of a particular kind of material.

FACTORS INVOLVED IN THE MAINTENANCE OF FORM

Degree of determination.—One of the primary factors concerned with the regulation of form appears to be the degree of determination. The differences in regulative capacity between the so-called "mosaic" eggs and "regulative" eggs can be attributed in part to this factor. It has been shown that certain substances or "factors" (Conklin, 1931, p. 77) in some of the so-called mosaic eggs, such as those of the scaphopod mollusk, *Dentalium* (Wilson, 1904), are determined for a particular rôle in development at the time of first cleavage. On the other hand, materials in some of the so-called regulative eggs, such as those of the sea urchin, *Echinus* (Driesch, 1891), are not irreversibly set for a particular end prior to the beginning of cleavage. The blastomeres of the former are potent for only a part of the whole; the blastomeres of the latter are totipotent. Earlier workers, especially Driesch, considered the materials in the early blastomeres A to D of the sea urchin to be indifferent. The part of the future mold which the stuffs of a given blastomere would fill, depends

on the position of the blastomere. The fact, however, that blastomere AB of the sea urchin will regulate and produce a whole larva, whereas the blastomere AB of *Dentalium* will form only a partial larva, does not preclude the possibility that substances in the zygote of the sea urchin are undergoing chemodifferentiation.

This has been strongly suggested by the experiment of Hörstadius (1928). If a zygote of the sea urchin is sectioned meridianally within the first half-hour after sperm entrance, the halves cleave according to the normal pattern, in the majority of instances. They produce, for example, the characteristic number of four micromeres at the sixteen-cell stage. If, however, the zygote is cut from thirty to sixty minutes after sperm entrance, the halves in an increasing percentage of instances cleave as if they were parts of a whole. Thus, after the fourth cleavage these halves possess either three or two micromeres. Although the pattern of cleavage has no effect on future development, since all halves produce whole larvae, Hörstadius' experiment indicates that in the sea urchin determination occurs prior to cleavage, as it does in *Dentalium*. Furthermore, the experiment indicates that determination is progressive: it begins imperceptibly, passes through a period of intensification, and finally attains a maximal intensity, which is characterized by a condition of irreversibility. In this the concept of determination differs from Lillie's idea of segregation (Lillie, 1929). It is not unlikely that other patterns, in addition to the pattern of cleavage, are determined in the early zygote of the sea urchin. They have not been demonstrated, however, because they are as yet labile and have not reached a state of irreversibility. A disturbing force may cause such patterns, which have not been irreversibly determined, to dissolve, as it were, and then reform later into new patterns.

Thus, the regulation of a particular pattern or part of a developing system occurs within a given period of development only, and that period coincides with the period of determination of the particular pattern or part. "The concept of regulation," says Spemann, "holds that an existing structure is changed or that a developmental process is directed into new channels" (Ruud and Spemann, 1922, p. 112). If this interpretation is accepted, we may consider that regulatory processes cannot occur prior to the initiation of determination.

When, on the other hand, either a field or a region becomes irreversibly determined, as we shall show, there is then no evidence of further regulation. The anteroposterior polarity of the amphibian forelimb field, for example, is determined during the period of gastrulation. At the time when the blastopore is crescentic in shape, presumptive forelimb materials have not as yet become irreversibly set with regard to polarity (Rotmann, 1931). If, however, presumptive forelimb mesoderm is removed from its position slightly lateral to the blastopore in a medium-sized yolk-plug stage and grafted with inverted orientation into an older embryo, a limb of reversed asymmetry will be formed (Detwiler, 1933). The field has now become irreversibly determined, at least with respect to its polarity along the anteroposterior axis, and limitations are henceforth placed on the regulative capacity of the field as a whole. A perfect

limb, however, may be formed from half of the limb field, or the entire field may form more than one normal limb, if a part be grafted into another region (Harrison, 1918). It appears, therefore, that a marked regulative capacity is still possessed by the regions within the limb field and that this capacity may be demonstrated until regional determination becomes irreversible. It might be added, however, that at a later period in the life cycle the organism may be capable of regulative activity, such as that associated with regeneration.

Physical conditions within the protoplasm.—In addition to the degree of chemodifferentiation, certain physical conditions appear to influence the extent of regulation, especially during the period of cleavage. Various experiments with isolated egg fragments and blastomeres, together with experiments in which materials within the egg have been stratified by means of a centrifuge, have shown that viscosity is certainly one of these physical factors. Conklin (1931, p. 71) states that "in general there is an inverse relation between the degree of [chemo-]differentiation and the lability of protoplasm, and there is also a direct relation between the latter and the power of regulation." For example, centrifugation has shown that the eggs of ascidians are composed largely of solid or semisolid material. Furthermore, it is known that this high viscosity may be accentuated by mechanical injury, such as the removal of parts of an egg or the separation of early blastomeres (Daleq, 1932). It is not unlikely, therefore, that the suppression of regulation in the early development of the ascidian (Conklin, 1905; Daleq, 1932) may be due to the physical rigidity of the cytoplasm.

An early experiment by Morgan (1895), in which he demonstrated that either a partial or a whole embryo may be produced from a single blastomere of the frog's egg, affords further evidence that physical factors play an important rôle in regulation. If one of the blastomeres of a two-cell stage be destroyed and the other blastomere be permitted to develop in its normal position, a partial embryo may be formed (Roux, 1888; Morgan, 1895). On the other hand, if the uninjured blastomere be inverted, a perfect embryo of one-half size may be produced (Morgan, 1895). Apparently, a pattern has been established in each of the first two blastomeres. This pattern may be for a lateral, a dorsal or ventral, or an oblique half of an embryo, according to the relation of the first plane of division to the plane of bilateral symmetry. The materials in a blastomere would be faithful to that pattern of early but reversible determination as long as the pattern should remain undisturbed. If the blastomere be inverted, a rearrangement of the materials takes place, the original pattern for a half embryo becomes obliterated, and a new one, for a whole, is reestablished. Schultze (1895) demonstrated that the pattern in both blastomeres of the frog's egg could be similarly altered by inverting the egg in the two-cell stage. These experiments show that the maintenance of a certain physical rigidity within the cytoplasm is probably essential for the maintenance of the original pattern of determination.

QUANTITATIVE AND QUALITATIVE ASPECTS OF REGULATION

The maintenance of specific organic form involves a regulation of (1) amount and (2) kind of material. From the stuffs at hand, regardless of abundance or paucity of materials, regulatory development tends to fill the mold of specific organic form in such a manner that unity and harmony will be preserved. Either of the factors considered above, however, may militate against regulation; in this event, the mold is filled only in part. Although both quantitative and qualitative regulation of form coexist and are distinguished only arbitrarily, certain experiments demonstrate the former aspect more clearly than the latter.

Regulative control of amount of material.—In the egg of the ctenophore, *Beroë*, Yatsu (1911) has demonstrated that if large portions of the cytoplasm are removed from the lower pole of the egg at the time of first cleavage, the micromeres, although correspondingly smaller, may be of correct proportions. Their size depends largely upon that of the fragments from which they are produced and not upon the amount of ectoplasm contained within the fragments.

A remarkable example of quantitative regulation is also provided by the experiment of Mangold and Seidel (1927), in which there is an addition instead of a loss of substance. Two eggs of *Triton* are fused together at the two-cell stage. There is more than the normal amount of material within the egg; furthermore, the arrangement of stuffs is abnormal, since the two eggs are fused so that their blastomeres alternate. A unitary and harmonic structure may result nevertheless, the embryo being single, whole, and of correct proportions.

Regulative control of kind of material.—The qualitative aspect of regulation is perhaps best exemplified in certain experiments in which portions of early gastrulae are fused together so that the composite embryo contains either less or more of a particular kind of material. These embryos have been designated by Spemann and Bautzmann as "Kleinkeime" and "Grosskeime," respectively. The former is produced by merely removing a median section from an early gastrula and then fusing the lateral portions together; the latter, by making a parasagittal section to the right of the median plane in one gastrula, and to the left of the median plane in another. The small lateral portions are discarded and the two larger parts fused together, thereby providing the composite embryo with more median material than is normally present. "In spite of deficiency or excess of materials, a normally proportioned embryo is produced. Some kind of regulation must therefore take place, and as a result either deficiencies are compensated for or excess materials are utilized for other purposes" (Spemann and Bautzmann, 1927, p. 576).

It is clear from the experiment of Spemann and Bautzmann just mentioned that the division of ectoderm into epidermis and medullary plate and of mesoderm into chorda and somites may be altered by experimental conditions. In the *Kleinkeime* the following qualitative changes appear to be necessary for harmonic disposition of the excess materials: (1) a certain amount

of presumptive epidermis becomes medullary plate, and (2) a certain amount of prospective somite material becomes chorda. In the *Grosskeime*, on the other hand, the qualitative regulation is reversed: (1) some presumptive medullary plate becomes epidermis, and (2) some prospective chorda becomes somite. It appears, therefore, that even mesodermal materials are not irreversibly determined at the beginning of gastrulation. We observe, however, that the regulation of chorda and somite anlagen has been within the chordamesoderm. If presumptive chorda could become medullary plate, then we should have an even more remarkable example of regulation.

STATEMENT OF THE PROBLEM

The formative movements which take place during the gastrulation of an amphibian egg are of vital importance in the establishment of the major organ systems. Thus, the processes of invagination, involution, and epiboly serve (1) to transport materials to definitive positions, (2) to convey organizing centers, such as those for the medullary plate, the eye, or the forelimb, to their respective sites of induction, and (3) to establish the primary pattern of embryonic form. An adverse force, however, may be experimentally created which will alter the normal mechanics of gastrulation and thereby cause materials and centers of organization to be carried to a foreign position. Such an untoward force may be obtained by filling the segmentation cavity of late blastulae with gelatin (Eakin, 1933). The regulatory development which then occurs provides a new avenue of approach to the problem of regulation.

The following experiments were performed in order to study more completely the general problem of regulatory development in *Triturus torosus*. In particular, an attempt was made to ascertain what factors are involved in the shifting of the medullary plate, the reorganization of prospective chordamesoderm, and the establishment of embryonic form in a foreign position.

This investigation was conducted under the direction of Professor J. Frank Daniel; to him I wish to express my deepest appreciation for his helpful suggestions and advice in the experimental work, and for his assistance in the preparation of this paper.

MATERIAL AND METHODS

For the most part the eggs of the Pacific Coast newt, *Triturus torosus*, were used. In some experiments, however, the eggs of the Pacific tree frog, *Hyla regilla*, were employed as donors of ectodermal transplants. Some of the material was collected directly from ponds and streams near Berkeley, California; for the most part, however, the eggs were obtained from animals which mated and spawned in the laboratory.

The method of injecting the blastocoele with gelatin has been reported in detail in an earlier communication (Eakin, 1933). Experiments in which vital stains were used followed the technique of Vogt (1925). Those involving transplantation were carried out as follows. After removal of the chorion from a blastula of *Hyla* as donor, transplants were obtained by extirpating a relatively large section from a given region of the embryo. This piece was sub-

sequently trimmed or cut into smaller squares or rectangles by means of a fine scalpel. A small incision was then made in the chorion of an early gastrula of *Triturus*, and a piece of material, slightly smaller than the transplant, was removed from a selected area of this embryo. Incisions were made by means of two needle knives, instead of the usual combination of knife and hair loop. A small glass rod which terminated in a minute knob was used to push the transplant through the incision in the chorion and to place it in the host. The transplant was usually held in position by the chorion.

When an incision is made in the chorion of an injected blastula for purpose of transplantation, internal pressure causes an extrusion of part of the embryo through the chorion. The incision gradually enlarges, thereby permitting more and more of the embryo to protrude through the chorion. Under these conditions gastrulation seldom takes place. Accordingly, transplantation was performed before injection. Care was taken to make the incision in the chorion no larger than the transplant. After the graft had healed in place, the embryo was rotated within the chorion until the incision in the membrane was brought over the animal pole. Injection could then be made through the same chorionic aperture as had been used for transplantation.

RESULTS

MORPHOLOGICAL FEATURES OF EXPERIMENTAL EMBRYOS

Before considering the factors involved in the regulatory development of embryos injected with gelatin, it is well to present first a description of their purely morphological features and to contrast experimental with normal embryos, particularly at the gastrula, neurula, and early larval stages. From a total of 399 specimens a few have been selected to illustrate these general features.

THE GASTRULA

When the cannula is withdrawn from the egg a certain amount of gelatin may remain between the surface of the egg and the chorion. This solidifies when the egg is transferred to cold water and apparently prevents an enlargement of the aperture made in the chorion for the purpose of injection. The protective rôle of this investing membrane, which in both normal and experimental embryos is of great importance during the period of gastrulation, is thus preserved.

Externally the experimental gastrula differs from the norm in the following particulars. Although the wound sustained by the roof of the blastocoele usually heals, there frequently persists a slight depression, which has been intensely stained by neutral red and which serves to mark the point of injection. As a result of the diffusion of vital dye from the mass of gelatin, the animal hemisphere becomes slightly stained. On dissection, the gelatin and inner surfaces of the ectodermal cells are found to be colorless. It appears that the vital dye is localized in the distal or outer ends of the cells.

That portion of the ectodermal surface which is underbedded by gelatin is further characterized by extensive wrinkling. The shallow furrows and

slightly raised convolutions resemble in general appearance the gyri and sulci of the human cerebrum. Specimens injected with an excessive amount of gelatin generally show more wrinkling than those which contain a smaller gel mass. Finally, a slight circumferential depression in the gastrula marks the boundary between the gelatin and the mass of yolk.

A gross dissection of the early gastrula discloses the gelatin as a hemispherical mass beneath the ectodermal cells. It is relatively rigid and adheres tenaciously to the ectodermal cells, and likewise to the yolk cells below. Apparently, the gelatin solution runs in among the cells at the time of the injection, but later solidifies and so holds the cells firmly to the central gel mass. One can easily make a circular incision at the equator to separate the animal and vegetative hemispheres. The former will maintain its hemispherical form because of the gelatin mass beneath, and may be allowed to continue development as an explant.

In a cross section (fig. A) of a very late experimental gastrula, *no. 1A6*, the ectodermal cap (*an.*) covering the gelatin (*g.*) is continuous over the vegetative hemisphere, and an early indication of the future medullary plate

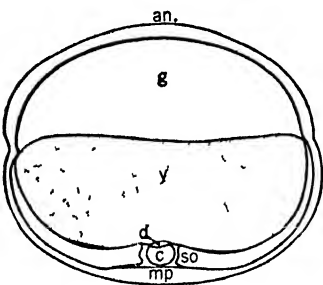


Fig. A. Transverse section of experimental late gastrula of *Triturus torosus*, *no. 1A6*. *an.*, epidermal cap; *g.*, gelatin; *mp.*, medullary plate; *c.*, chorda; *so.*, somite; *d.*, digestive tract; *y.*, yolk cells.

(*mp.*) may be seen in the thickened median portion of the ventral ectoderm. Above this we observe the chordamesoderm, which has already formed as notochord (*c.*) and somites (*so.*). Immediately above the notochord is the diminutive archenteron (*d.*), exceedingly narrow and flattened in contrast with that of a normal gastrula. This feature of the experimental embryo will be considered later. Central to the archenteron and the mesodermal somites is the compact body of yolk cells (*y.*).

If the blastocoele is distended, the pressure of the gelatin within prevents the closure of the aperture through which the cannula was inserted. This aperture then gradually enlarges until a complete view of the interior is possible. Observed through the transparent gelatin, the size and disposition of the yolk cells can be clearly ascertained. Many gastrulae which have been filled with an excessive amount of gelatin form numerous apertures in the ectodermal layer in addition to the single one near the animal pole. These openings are small at first; later they gradually enlarge in one or more directions until some of them form extensive windows. Figure 4 (pl. 24), drawn forty-eight hours after injection, represents an example, *no. 10A6*, which is extreme in this regard. The ectodermal cap (*an.*), gelatin (*g.*), and yolk cells (*y.*) may be clearly seen. In this example, the blastocoele was forcibly distended at the time of injection, so that the ectodermal cap was subjected to considerable internal pressure. During the period of gastrulation there is a constriction of the marginal zone (*e.*) which produces tension upon the ectodermal cells. In the norm this tension may be relieved by decreasing the size of the blastocoele, but in the experi-

mental gastrula this is precluded by the mass of gelatin. It seems, therefore, that this tension, together with the internal pressure, ultimately causes masses of ectodermal cells to separate here and there, thus giving rise to the apertures.

The ectodermal cells around the margins of each aperture tend to curl under for a short distance, forming an internal stained layer. This is easily discerned, because the outer pigmented and stained surfaces of the cells then become involuted to form the inner surfaces. It has been stated above that the inner surface of the single layer of cells is colorless. The several apertures increase in size until their margins are separated only by cylindrical strands or cords, some of which are several cells in thickness. Increased tension may then cause certain of these cords to break and two windows to become confluent.

THE NEURULA

Morphologically the experimental neurula differs markedly from the normal neurula. Figure 2 (pl. 24) shows an injected embryo, *no. 1D4*, which has been rotated to show the vegetative area (*vg.*). First, it will be seen that the medullary plate anomalously occupies a position across the vegetative hemisphere, whereas normally it lies across the animal hemisphere (see pl. 24, fig. 1). Schechtman (1932) has shown that in the norm the region of the animal pole in *Triturus torosus* marks the general location of the transverse fold of the medullary plate. Hence the transverse fold of the experimental neurula is situated approximately 90 to 110 degrees below its normal position. This will be considered at greater length in discussion of the movements of materials.

In addition to the position of the medullary plate, the experimental neurula differs from the norm in size, shape, and general appearance of the medullary plate. The plate of the injected embryo is characteristically narrow even at the beginning of neurulation. In shape the future brain area is relatively small and oval, and the spinal region is long and slender. Furthermore, irregularities in the medullary plate are commonly observed in experimental neurulae. As examples, the brain area may show marked asymmetry and the region of the future spinal cord may be wavy, as seen in figure 2, or it may even be bent at right angles. On the other hand, the control possesses a broad medullary plate the lateral borders of which have migrated up from almost the level of the equator. In general shape it is not unlike a tennis racket, with a broad anterior part, the brain area, and a relatively short and wide stalk, the spinal region.

The experimental neurula is further distinguished from the control by a smaller amount of pigment and by the character of the neural folds. The pigment which is present, however, is distributed as in the norm, that is, with the greatest concentration along the margins of the medullary plate. The neural folds are narrow and exceedingly low and flat (see pl. 24, fig. 2); in fact, in the region of the spinal cord they are frequently absent. Here the medullary plate sinks in from the surface as a shallow groove which eventually closes over at the end of neurulation. In the cephalic area, however, the folds are slightly raised ridges which gradually approximate and fuse, much as in the

control. A gross dissection of the medullary plate of an experimental neurula (see Eakin, 1933, pl. 14, fig. 4) reveals the underlying layer of chordamesoderm, which can be followed readily along its entire length under the medullary plate.

THE EARLY LARVA

An experimental embryo, no. 10C12 (see fig. 3, pl. 24), will be described to illustrate the essential features of the early larva. Although the relation of the

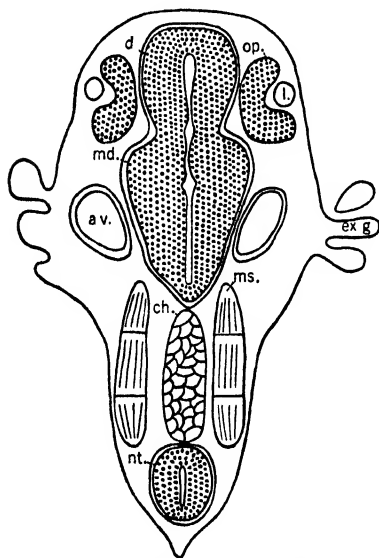


Fig. B. Frontal section of experimental larva of *Triturus torosus*, no. 10C12. *d.*, diencephalon; *md.*, medulla; *op.*, optic cup; *l.*, lens; *av.*, auditory vesicle; *ex.g.*, external gill; *ch.*, chorda; *ms.*, muscle segments; *nt.*, neural tube.

embryo to the axis of the egg has changed little since the neurula stage, the axial organs have elongated considerably, thus bringing the head and tail upward about the bubblelike epidermal cap which covers the gel mass. The swelling (*op.*) on the side of the head indicates the position of the eye; the three external gill rudiments (*ex. g.*) and the forelimb bud (*fl.*) may be observed in their approximately normal positions. Although a balancer is wanting in this specimen, it was formed in others. Embryos were not reared further than this stage.

An oblique section (fig. B) through the head of the specimen described above reveals the degree of internal differentiation in some of the major organs. The chief divisions of the brain, of which only the diencephalon (*d.*) and medulla (*md.*) are here shown, have clearly differentiated. Optic cups (*op.*), lenses (*l.*), well-formed auditory vesicles (*a. v.*), notochord (*ch.*), and muscle segments (*ms.*) are also observable. All specimens, however, did not show so complete and orderly a differentiation as this one.

MOVEMENT AND LOCALIZATION OF PRESUMPTIVE MATERIALS

EVIDENCE FROM VITAL STAINING

In the norm.—A series of preliminary experiments was performed to determine if the map of presumptive areas in the blastula of *Triton* (Vogt, 1929) holds likewise for *Triturus*. Schechtman (1932, 1934) has shown that the boundary between prospective epidermis and presumptive medullary plate in *Triturus* agrees essentially with that in *Triton*, and I have confirmed this observation. The line of demarcation between the medullary anlage and presumptive chordamesoderm, however, appears to be slightly lower in the blastula of the Pacific Coast newt (Schechtman, 1934, pl. 22, fig. 1) than in the European newt. The following experiment verifies this point and shows more completely the fate of various regions above the dorsal lip of the blastopore.

On a control specimen, no. 2M2, a pattern of vital stains (fig. C, 1) was made above the dorsal lip at the beginning of gastrulation. Mark 1 was placed immediately above the blastoporal groove (b.); mark 2 lay in the median plane approximately midway between the blastopore and the equator (e.) of the egg; marks 4 and 5 were lateral to mark 2; whereas the position of mark 3 was just below the equator and in line with stains 1 and 2.

Figure C, 2 represents the position of the stained areas in the midgastrula stage. Mark 1 has completely disappeared within the circular blastopore (b.). Marks 2, 4, and 5 have been stretched toward the blastopore, and the lower portions of the stained areas have been carried inside. Mark 3 likewise shows stretching toward the blastopore.

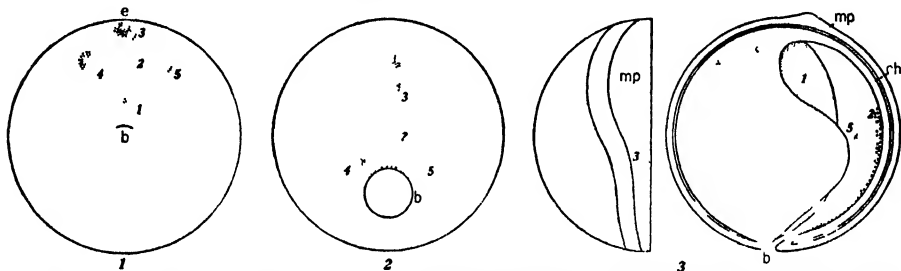


Fig. C. 1. Location of stains (1-5) in normal early gastrula of *Triturus torosus*, no. 2M2, posterior view. b., blastopore; e., equator.

2. Location of stains (2-5) in normal midgastrula of *Triturus torosus*, no. 2M2, posterior view. b., blastopore.

3. Location of stains (1-5) in normal neurula of *Triturus torosus*, no. 2M2, bisected, with the right half placed on its side to show the position of vital marks internally. b., blastopore; ch., chorda; mp., medullary plate.

The location of vital marks in the neurula stage may be observed in figure C, 3. The embryo has been bisected through the median plane and the right half placed on its side so as to indicate the position of marks internally. Mark 1 now lies on the forewall of the archenteron, the major portion of it lining the entodermal floor of the future foregut. Mark 2 has been carried entirely inside during gastrulation and now extends as a long narrow stripe on the median longitudinal part of the archenteric roof (chorda, ch.), from approximately the level of the future auditory vesicle almost to the blastopore (b.). Mark 5 occupies a lateral position parallel with mark 2 and extends along the right border of the chorda and on the mesoderm beneath the right half of the medullary plate (mp.). Mark 3, however, has remained on the outside (left half of fig. C, 3) and now lies on the posterior portion of the medullary plate.

From the definitive positions of these vital marks it is shown that the boundary separating presumptive chordamesoderm and the medullary anlage in the norm lies between marks 2 and 3 (fig. C, 1). Thus, in *Triturus*, the material which forms the dorsal rim of the definitive blastopore is situated slightly below the equator of an early gastrula. In *Triton*, on the other hand, this presumptive dorsal rim material is situated at or even above the equator. This study shows, further, that the material immediately above the dorsal lip of *Triturus* is destined to form the anterior wall of the archenteron, as it does in *Triton*.

In the experimental embryo.—A pattern of vital stains (fig. D, 1) similar to that in the preceding study was placed above the blastoporal groove (*b.*) of an early experimental gastrula, no. 2N5. The blastocoel of the gastrula was then filled with gelatin and the positions of the marks recorded. A series of marks (1 to 3) lying in or near the sagittal plane extended from the blastopore to the equator (*e.*); marks 4 and 5 were lateral in position and on the left and right sides, respectively; mark 4 was approximately at the same level as mark 2; mark 5 lay immediately below the equator.

Mark 1, which at the beginning of gastrulation lay just above the blastoporal groove, was no longer visible at the middle gastrula stage (fig. D, 2).

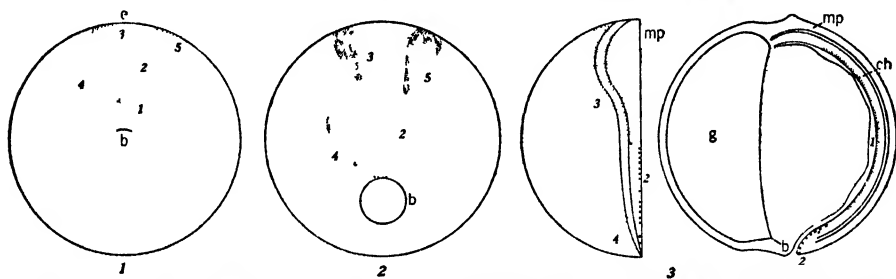


Fig. D. 1. Location of stains (1-5) in experimental early gastrula of *Triturus torosus*, no. 2N5, posterior view. *b.*, blastopore; *e.*, equator.

2. Location of stains (1-5) in experimental midgastrula of *Triturus torosus*, no. 2N5, posterior view. *b.*, blastopore.

3. Location of stains (1-4) in experimental neurula of *Triturus torosus*, no. 2N5, bisected, with the right half placed on its side to show the position of vital marks internally. *g.*, gelatin; *b.*, blastopore; *mp.*, medullary plate; *ch.*, chorda.

The remaining marks showed extensive stretching, particularly mark 2. The lower portion of mark 4 had almost reached the large circular blastopore (*b.*).

With the advent of the medullary plate (*mp.*, fig. D, 3) it is to be observed that the greater part of mark 3 lies on the left side within the confines of the future brain region and the anterior portion of the spinal cord. Mark 5, not shown in the figure, occupies a similar position on the right side. Thus, the lateral extent of the presumptive brain area is narrower in the experimental than that in the normal urodele egg (Goerttler, 1925; Schechtman, 1934). Most of mark 2 extends superficially along the medullary plate to the blastopore; some of it, however, has been carried inside and now lies on the extreme posterior portion of the archenteric roof. This may be observed in the right half of figure D, 3. The posterior end of mark 1, which lies also in the median line of the archenteric roof, may be observed likewise immediately anterior to mark 2. Mark 1 may then be traced forward to the level of the future diencephalon. The major portion of mark 4 lies outside the blastopore, on the caudal portion of the medullary plate and the adjoining epidermis (left half of fig. D, 3).

From this study of an experimental embryo we see that chorda has been derived only from material lying between the lower border of mark 1 (fig. D, 1) and the lower part of mark 2. In the control, on the other hand, presumptive chorda always extends beyond the upper border of mark 2 (fig. C, 1). Thus, in the control all of the material so stained was rolled in during gastrulation

and became chorda; although this region is a relatively small area at the beginning of gastrulation, we note that it is greatly stretched and ultimately forms the greater part of the notochord. In the experimental embryo, however, only a small part of the ventral border of mark 2 formed chorda. The question, then, is, What has been the fate in the experimental embryo of the dorsal part of the normal chorda anlage? We observe that the greater part of mark 2 (fig. D, 1) eventually lies in the region of the medullary plate which will form the spinal cord. Therefore the entire dorsal part of the chorda anlage has become *medullary plate* and has not differentiated into notochord according to its prospective value. This conclusion is based upon a study of more than one hundred examples, from which the one just described was selected.

Vogt (1922) and Mangold (1923) early demonstrated that under certain conditions presumptive mesoderm, particularly that from the ventral one-sixth of the germinal ring (Mangold, 1923, p. 296), may differentiate into ectodermal structures. Evidence that even more dorsal portions of the germinal ring can regulate was provided by the experiments of Bruns (1931). In these a considerable amount of ectodermal material in *Triton* or in *Bombinator* was removed before gastrulation. The closure of the wound was, in some instances, partly effected by presumptive chordamesoderm, which then remained on the outside and subsequently differentiated into ectodermal structures. In these instances it was noted, further, that the mesodermal organs on the side of the embryo sustaining the wound were, as a result of sacrifice of material, not so well developed as those on the unoperated side. The experiments of Holtfreter (1931) and Schechtman (1934), employing explantation, have further indicated that the chordamesoderm is not irreversibly determined at the beginning of gastrulation.

Since the preliminary paper (Eakin, 1933) on the present work appeared, Lopaschov (1935) and Töndury (1936) have contributed additional evidence of the regulative capacity of the dorsal lip region in *Triton*. The former replaced a piece of the medullary plate with dorsal lip material from an early gastrula. In a few instances the presumptive chordamesoderm differentiated into unmistakable neural tissue. The latter interchanged autoplastically the dorsal lip area of an early gastrula with presumptive medullary plate, with prospective epidermis, or with presumptive ventral mesoderm. In all three types of exchange the presumptive chordamesoderm may develop according to the site of transplantation. The highest percentage (79 per cent) of instances showing regulation was obtained in the first type of interchange, that is, an exchange between presumptive chordamesoderm and prospective medullary plate. Töndury believes that the essential factor in this regulation is the suppression of the formative movements of the transplanted chordamesoderm (p. 108). If these dynamic movements are not overcome, the transplant will differentiate according to its prospective fate and not in accordance with its new position.

As for injected embryos, what has been the effect of the experimental procedure upon the formative movements taking place during gastrulation? The blastocoele which normally accommodates the invaginating entoderm and the

inrolling chordamesoderm is already occupied by gelatin in the experimental gastrula, and typical invagination and involution can, therefore, proceed from the position of the blastoporal groove for only a short distance to the base of the gel mass. The advancing yolk floor of the archenteron and the layer of inrolling chordamesoderm move in the direction of the animal pole as gastrulation proceeds. At approximately the level of the equator, however, these two advancing layers meet the base of the gel mass. The resistance of the gelatin apparently prevents a further anterior migration of the archenteron. The definitive position of the anterior end of the archenteric roof, that is, the chordamesodermal layer, is thus at the base of the gel mass.

Gastrulation having proceeded as far as the experimental conditions permit, the processes of invagination and typical involution are thereafter precluded. The overgrowth of the cells at the dorsal blastoporal lip, however, is

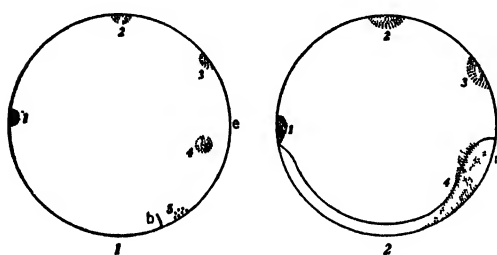


Fig. E. 1. Location of stains (1-6) in experimental early gastrula of *Triturus torosus*, no. 2F10, side view. b., blastopore; e., equator.

2. Location of stains (1-4) in experimental neurula of *Triturus torosus*, no. 2F10, side view. mp., medullary plate.

not interfered with. In fact, overgrowth becomes greatly accentuated, since the only other alternative course to be taken by these cells, which cannot pass inward, is migration downward over the vegetative area. The dorsal lip of the blastopore gradually advances over the yolk and may even reach the rim of the ventral lip. At the same time, however, the marginal cells of the dorsal lip are turned under to form

the roof of the archenteron. This process has been termed "modified involution" (Eakin, 1933) in contradistinction to true involution, in which the archenteric roof (chordamesoderm) moves anteriorly and gradually encroaches upon the blastocoel. These two processes of overgrowth and modified involution in experimental gastrulation may be likened to a tractor which rolls the upper tread under as the tractor advances.

Since the heavy yolk-laden cells of the vegetative hemisphere cannot be displaced into the segmentation cavity, the center of gravity of the embryo remains unchanged, and rotation does not occur. The following experiment shows this point and adds further information concerning the fate of certain areas of the ectodermal cap.

Three marks (1-3, fig. E, 1) were placed on the ectoderm and in the sagittal plane of an early experimental gastrula, no. 2F10. Mark 1 lies immediately above the equator on the ventral side; mark 2 may be seen in the region of the animal pole; and mark 3 has been placed about midway between the center of mark 2 and the equator (e.) of the egg. Marks 4 and 5, on the other hand, are below the equator. Mark 5 is above the blastoporal groove (b.) and in the median plane, whereas mark 4 lies lateral to mark 5 and on a higher level. Following the staining, the embryo was injected.

In the neurula stage (fig. E, 2) it is to be observed that marks 1, 2, and 3 have not changed their relative positions, although all now cover slightly greater areas. Vital mark 5 has been carried into the blastopore (b.), and mark 4 has been stretched so that most of it now lies within the anterior part of the medullary plate (mp.).

It is to be observed in this experiment that the animal hemisphere, or ectodermal cap above the gelatin, becomes epidermis only. It is known, however, that the ectoderm in the norm is almost equally divided into the epidermal and medullary anlagen (Vogt, 1929). The approximate boundary separating these two presumptive areas in *Triturus* (Schechtman, 1932) is represented

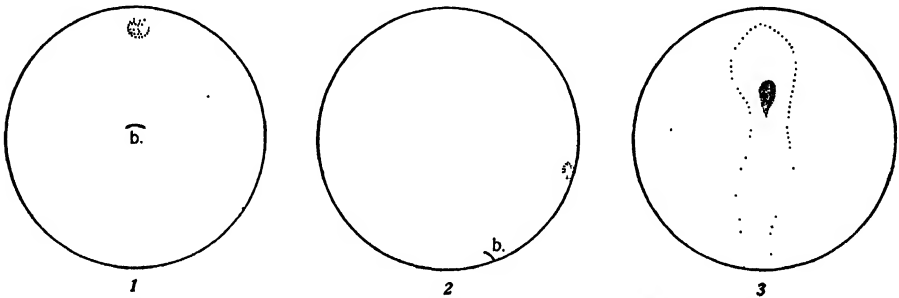


Fig. F. 1. Experimental early gastrula of *Triturus torosus*, no. 3DS, showing the location of the transplant (stippled) from *Hyla*, posterior view. b., blastopore.
 2. Experimental early gastrula of *Triturus torosus*, no. 3DS, showing the location of the transplant (stippled) from *Hyla*, side view. b., blastopore.
 3. Experimental neurula of *Triturus torosus*, no. 3DS, showing the location of the transplant (stippled) from *Hyla*, dorsal view.

in the early gastrula by the region of the animal pole (mark 2, fig. E, 1). Thus, all the material lying between the center of mark 2 and the lower border of mark 3 would form medullary plate in the norm; in the experimental embryo, however, the same region becomes epidermis. From this we see that certain materials in the experimental embryo do not differentiate according to their prospective values. Some investigators, namely, Goerttler (1927), Lehmann (1928), and Erdmann (1931), have maintained that ectodermal materials are allocated for particular purposes even at the beginning of gastrulation. For example, the medullary anlage is regarded as possessing some self-differentiative capacity even when isolated from chordamesoderm. It is shown in the present paper, however, that the greater part of the presumptive medullary plate becomes epidermis. These results are in agreement with those obtained by Holtfreter (1933b) in his experiments in exogastrulation. Therefore, any pattern of early determination existing in the medullary anlage would be obliterated by the regulatory development which takes place during experimental gastrulation, and a new pattern established for epidermis.

EVIDENCE FROM TRANSPLANTATION

The evidence presented above concerning the fate and movement of materials in experimental gastrulation may be tested by means of transplantation. Instead of marking a given area with a vital stain, one may transplant into that

region material which differs in some visible character from that of the host. Thus, a piece of material from a pigmented egg may be transplanted into a particular area of a lighter-colored egg and the transplant, by virtue of its darker color, may be followed during subsequent development. European workers generally have employed reciprocal transplants between two (or more) species of *Triton* the eggs of which differ in amount of pigmentation.

It was found, in this investigation, that ectodermal transplants from a middle blastula of *Hyla regilla* could be grafted easily into the early gastrula of *Triturus torosus*. The transplant, because of its darkly pigmented and smaller cells, can be identified at any later stage in development, and the fate of the presumptive area into which the graft was made may be judged, therefore, from the definitive position of the transplant. Eighty-nine such xenoplastic transplantations were performed; in twenty-three of them the transplantation was successful and the host was maintained until at least the neurula stage. Only two representative instances will be described.

An ectodermal transplant from a blastula of *Hyla regilla* was placed above the blastoporal groove (b., figs. F, 1 and F, 2) of an early experimental gastrula, *Triturus*, no. 3D3. The transplant thus lay on the upper border of the normal chorda anlage (see fig. C, 1). After the graft had healed in place, the embryo was injected with gelatin. In the neurula stage (fig. F,

3) the transplant lay in the center of the future hind brain. A cross section of this early neurula (fig. G, 1) at the level of the graft showed the transplant (in stipple) in the median line of the medullary plate (mp.), underbedded by the chordamesodermal layer (cm., fig. G, 1) of the host.

A transplant had been similarly placed in a control, no. 3E3, at the beginning of gastrulation. By the middle of gastrulation the transplant had been carried to the margin of the blastopore, then circular in shape. It has been stated above that the transplant in the experimental gastrula showed no change in position at this time. In the control, however, the transplant by the close of gastrulation had reached the blastopore and had rolled in to form part of the posterior archenteric roof. The movement of the transplant, therefore, agrees with the movement of the upper border of vital mark 2 (fig. C, 1) in the control, no. 2M2. Likewise, the behavior of the transplant in the experimental embryo was similar to that of the material between the lower part of mark 3 and the upper border of mark 2 (fig. D, 1) in the experimental gastrula, no. 2N5.

The evidence from both vital staining and transplantation shows, therefore,

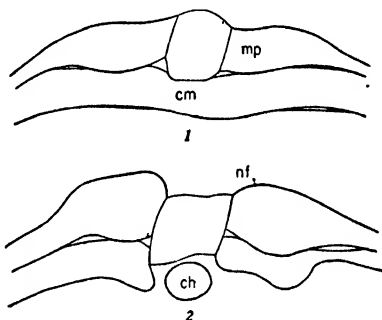


Fig. G. 1. Cross section of the dorsal organs of early experimental neurula, *Triturus torosus*, no. 3D3, at the level of the transplant (stippled) from *Hyla*. mp., medullary plate; cm., chordamesoderm.

2. Cross section of the dorsal organs of experimental neurula of *Triturus torosus*, no. 3D4, at the level of the transplant (stippled) from *Hyla*. nf., neural fold; ch., chorda.

that the dorsal part of the normal chorda anlage becomes medullary plate. As with the upper border of vital mark 2 (fig. D, 1), the transplant just mentioned was placed within presumptive chorda; in the norm it would have been carried inside during gastrulation and would have ultimately formed notochordal tissue. Under experimental conditions it does neither. It remains outside and lies within the medullary plate. Thus, the fate of material lying between the caudal border of the transplant (fig. F, 3) and the slit-shaped blastopore has been changed from one of skeletal to that of nervous tissue.

It is noteworthy that although the transplant in the experimental embryo remained unaltered in position, it changed in shape. The piece of material from *Hyla*, originally square, after healing became more or less circular. In

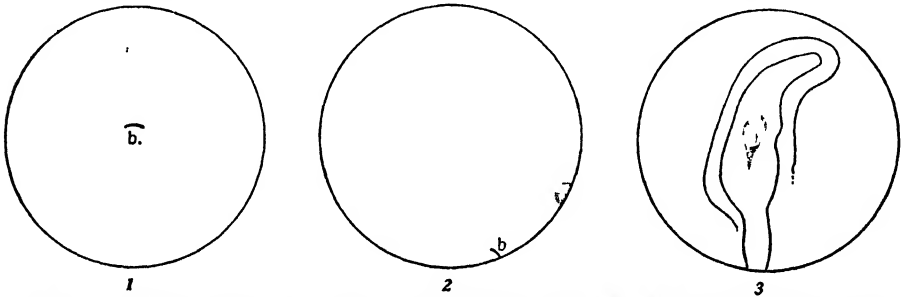


Fig. H. 1. Experimental early gastrula of *Triturus torosus*, no. 3D4, showing the location of the transplant (stippled) from *Hyla*, posterior view. b., blastopore.

2. Experimental early gastrula of *Triturus torosus*, no. 3D4, showing the location of the transplant (stippled) from *Hyla*, side view. b., blastopore.

3. Experimental neurula of *Triturus torosus*, no. 3D4, showing the location of the transplant (stippled) from *Hyla*, dorsal view.

the neurula stage, however, the graft had become elongated in the antero-posterior direction of the medullary plate and had become wedge-shaped at its posterior end. As the dorsal lip advanced over the vegetative area by means of overgrowth, the regions above the rim of the blastopore likewise proliferated, although not so rapidly. As a result of this proliferation the cells of the host adjoining the graft moved slightly toward the blastopore and carried with them the lower cells of the transplant.

A second experimental embryo, no. 3D4 (fig. H, 3), shows the final position of the *Hyla* transplant placed in the region of normal presumptive chorda (figs. H, 1 and H, 2) at the time when the blastoporal groove (b.) was just forming. Since the transplant in this experimental embryo was nearer the blastopore than the graft in the preceding experiment, it follows that the definitive position of the graft should be caudal to the brain area. We observe that the transplant ultimately lies within the region of the future spinal cord. Moreover, the elongation of the transplant is even more pronounced in the example just mentioned (fig. H, 3) than in the one in which the graft was made at a higher level above the blastoporal groove, indicating that the degree of proliferation becomes greater toward the dorsal lip. A cross section of the dorsal organs (fig. G, 2) at the level of the graft shows the transplant immediately above the notochord (ch.) and between the neural folds (nf.), which were well formed at the time the embryo was fixed (fig. H, 3).

The behavior of this transplant may be compared with that of the upper part of vital mark 2 (fig. D, 1) of the experimental gastrula, *no. 2N5*. Both the vital mark and this transplant lay entirely and unmistakably within the area of presumptive chorda. This region in the norm becomes the posterior part of the archenteric roof, that is, notochord (figs. C, 1-C, 3). Under experimental conditions, however, both the vital marks and the transplants remain outside the blastopore and in the region of the future spinal cord. The evidence from transplantation, therefore, confirms that obtained from vital staining (p. 196); both show that in the experimental gastrula materials do not differentiate according to their prospective value, and that the most striking qualitative change is, as has here been shown, the transformation of presumptive chorda into medullary plate material.

SUMMARY AND CONCLUSIONS

1. This investigation has attempted to analyze the problem of regulation in the Pacific Coast newt, *Triturus torosus*, particularly with respect to the chordamesodermal and medullary anlagen.

2. The normal mechanics of gastrulation were altered and regulatory development thereby initiated by injecting gelatin into the segmentation cavity of the late blastula or early gastrula (Eakin, 1933).

3. The mechanical obstruction of the mass of gelatin modified the formative movements in gastrulation so that typical invagination and involution took place only during the early part of gastrulation. After the advancing archenteron reached the base of the gel mass, further anterior migration of both the yolk floor and the chordamesodermal roof of the archenteric cavity was precluded. Experimental gastrulation was accomplished largely by epiboly and by a process termed "modified involution." Rotation did not take place.

4. The primary results were: (1) The area of the dorsal lip migrated ventrally over the yolk and folded under, producing a deeper and a superficial layer. The deeper layer was formed of presumptive chorda; the superficial layer, of presumptive chorda and presumptive medullary plate. (2) The deeper layer, or lower part of the chordamesodermal anlage, induced the superficial layer, or upper part of the presumptive chorda material and posterior portion of the medullary anlage, to form medullary plate in a totally new position, across the vegetative area.

5. The movements of presumptive materials were followed by means of vital stains and transplanted pieces of ectoderm from the midblastula stage of *Hyla regilla*. From the definitive positions of the vital marks and transplants the prospective values of materials in the experimental embryo and the control were ascertained.

6. From this study it is concluded that certain materials in the experimental embryo do not differentiate according to their prospective values in the norm. The dorsal part of the chorda anlage becomes medullary plate, and the greater part of the medullary anlage becomes epidermis.

7. This investigation lends further support to the contention that without an organizing substratum (inductor) no medullary plate forms.

8. It was shown that the entire ectodermal cap, which is underbedded by gelatin, forms epidermis only. This is in agreement with the results of Holtfreter (1933a, b). If the ectodermal materials are allocated for particular purposes at the beginning of gastrulation, as some investigators believe, then the regulation which takes place during experimental gastrulation obliterates the pattern of early determination and establishes a new pattern.

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EXPLANATION OF PLATE

PLATE 24

Fig. 1. Normal neurula of *Triturus torosus*, view of left side. *mp.*, medullary plate; *nf.*, neural fold.

Fig. 2. Neurula of experimental *Triturus torosus*, no. 1D4, rotated to show the vegetative hemisphere. *an.*, epidermal cap; *mp.*, medullary plate; *vg.*, vegetative hemisphere.

Fig. 3. Larval stage of experimental *Triturus torosus*, no. 10C12, view of left side. *op.*, eye; *ex.g.*, external gills; *fl.*, forelimb bud.

Fig. 4. Gastrula of experimental *Triturus torosus*, no. 10A6, the blastocoele of which was forcibly extended at the time of injection. *an.*, epidermal cap; *g.*, gelatin; *e.*, equator; *y.*, yolk cells.



**A PULSATING CIRCULATION
APPARATUS FOR TISSUE CULTURES,
EMBRYOS, AND SMALL ORGANS**

**BY
J. A. LONG**

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A PULSATING CIRCULATION APPARATUS FOR TISSUE CULTURES, EMBRYOS, AND SMALL ORGANS

BY

J. A. LONG

(Preliminary communication)

FOR THE STUDY under the microscope of tissue cultures, eggs and embryos of mammals (and other animals), and small organs, while they are being bathed in a pulsating nutritive fluid, the simple apparatus of glass described below has been devised. Its general appearance and most of its essential features are shown in the photograph (pl. 25).

It is made of three (or four) pieces of plate glass (ultimately of pyrex), one thick, two thin, in which are drilled several holes for bolts. The reservoirs and specimen chamber are large holes cut through the uppermost thick glass. The valve chambers are recesses cut in the lower side of the upper plate. The reservoirs and the several chambers are connected with each other by means of very small holes drilled through the middle (thin) plate, together with grooves cut in the undersurface of the same middle plate. When the plates are bolted together and sealed, and covers are sealed over the reservoirs and specimen chambers, the small holes, grooves, and large holes, or chambers, constitute a closed system through which fluid can be circulated and kept sterile. The apparatus can be disassembled for cleaning.

Movement of the fluid is brought about by the rhythmic compression and release of a small piece of rubber tubing, closed at its upper end, connected with the upper part of one of the valve chambers or with the channel between valve chambers. The elasticity of the tube on release produces the suction. Movement of fluid in one direction only is controlled by the two valves, which consist of small disks of plate glass that cover the holes entering the centers of the valve chambers from below. Fluid enters through these central openings and escapes through peripheral apertures. The compression and release of the compression tube has essentially the same action as a piston in a cylinder, and with the alternating pressure and suction from the compression tube the valves open and close in the same way as in a pump. Compressions of the tube are produced by the action of an electromagnet which is operated by an interrupted direct current controlled by a pendulum, so that the rate of pulsation can be varied at will. Provision is also made for controlling the speed of flow or the amplitude of the pulsations. The pump is capable of producing a pressure of 300 mm. of mercury.

A filter can be introduced at any place in the suction line between reservoir and valves to protect the valves from large particles. In the apparatus illustrated it is a disk of felt in a recess in the floor of the outlet reservoir.

For flushing the valves without opening the system, rubber tubes are connected with the reservoir chambers; and fluid can be drawn off, as occasion demands. The tips of the short glass tubes by which the rubber tubes make their several connections with the system are tapered and fit accurately into tapered holes. In this same manner the canula is fitted into the inlet opening of the specimen chamber.

The canula is provided for the attachment of a blood vessel to the system for the perfusion of an embryo or organ. It can be made of any size and form, and is easily attached and removed. Fluid escaping from the perfused structure passes into the specimen chamber and on into the inlet reservoir.

The reservoir is in two parts for simplicity in construction and to increase the area for oxygen absorption. According as its size is smaller or larger, the apparatus can operate on a few cc. of fluid or on a large quantity. Oxygen absorption can be increased by greater reservoir area or by an accessory pump for spraying or circulating the fluid within the reservoirs. Provision is easily made for changing either the gas or the fluid in the reservoir.

The whole apparatus (without the magnet and compression mechanism) can be sterilized, and, except for a small amount of fluid that oscillates up and down in the compression tube and its connecting duct, the circulating fluid is in contact with glass only.

Each piece of apparatus is placed in its own small constant-temperature box.

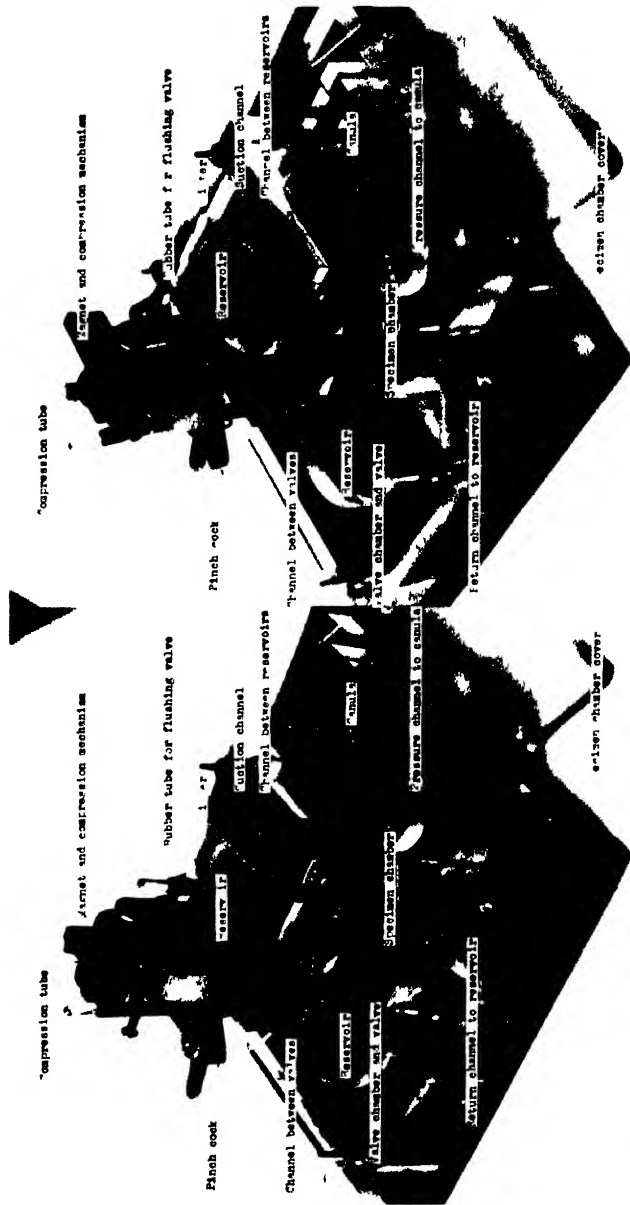
While the machine has already a large number of different designs of form and size (the number is almost unlimited), the basic part of the apparatus (all but the specimen chamber) is being standardized in a very few forms so that specimen chambers of any form and size can be attached according to need. The specimen chamber can be designed for any object from a single egg cell to a large embryo or small organ, and by enlarging all parts an object of greater size can be accommodated.

Other features and details of construction and of operation are reserved for later communication.

EXPLANATION OF PLATE

PLATE 25

A pulsating circulation apparatus for tissue cultures,
embryos, and small organs. $\times .6$.



**ORGANOGENESIS IN THE GASTEROPOD
CREPIDULA ADUNCA SOWERBY**

BY

C. E. MORITZ

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ORGANOGENESIS IN THE GASTEROPOD CREPIDULA ADUNCA SOWERBY

BY

C. E. MORITZ

INTRODUCTION

SURPRISINGLY LITTLE is known of the organogenesis of mollusks. Among the gasteropods, only one complete life history is known, that of *Viviparus (Paludina)* (Erlanger, 1891; Drummond, 1902; and others). Recently, Smith (1935) has given a fairly complete presentation of the development of *Patella*, a scutibranch. The present paper describes the development of the pectinibranch, *Crepidula adunca*, supplementing the account of the cell lineage of *C. fornicata* published by Conklin (1897). Since *C. fornicata* is not available on the Pacific Coast, *C. adunca* has been used in its stead. There seems little reason to doubt, however, that in the essentials of their development the two species are in agreement.

As with the paper on the anatomy of the adult (Moritz, 1938), much credit is due Professor S. F. Light, of the University of California.

MATERIAL AND TECHNIQUE

Crepidula adunca is protandrous. It is easily obtained along the northern California coast, where it breeds the year round. The nonmotile female lays the eggs in brood capsules on the "host" shell, usually *Tegula*. The young remain in the capsules through the young adult stage (figs. 8-11), all individuals in one capsule being approximately in the same stage of development. Material was obtained at Moss Beach, San Mateo County, and at Jenner, Sonoma County, California.

It was found best to remove the larvae from the capsules and to separate them before fixation. Zenker's or Kleinenberg's fixative gave good results. With paraffin sections, yolk offered little difficulty when the dioxan method was followed. Ten-micron paraffin sections were stained with Delafield's hematoxylin and eosin. Fifty- to 200-micron celloidin sections, prepared by Walls' method (1932), were stained with Delafield's hematoxylin or alum carmine, and those stained with alum carmine were counterstained with Lyon's blue.

THE EARLY EMBRYOLOGY OF CREPIDULA

The development of *Crepidula adunca* and *Crepidula fornicata* is the same as far as the 52-cell stage (Conklin, 1897, p. 22). The number of ectodermal cells then increases in *C. adunca* more rapidly than in *C. fornicata*. Larvae of *C. fornicata* have not been available for study, but, judging from Conklin's figures, the external resemblance between the larvae of the two species ob-

tains through the stage of shell-gland invagination (cf. fig. 1, and Conklin, 1897, pl. 7, figs. 75-77). This study begins, therefore, with the stage of shell-gland invagination. Various stages in the development of *C. adunca* are shown in figures 1 to 8 inclusive. Since a great part of the external development is either illustrated by these figures or is discussed under the develop-

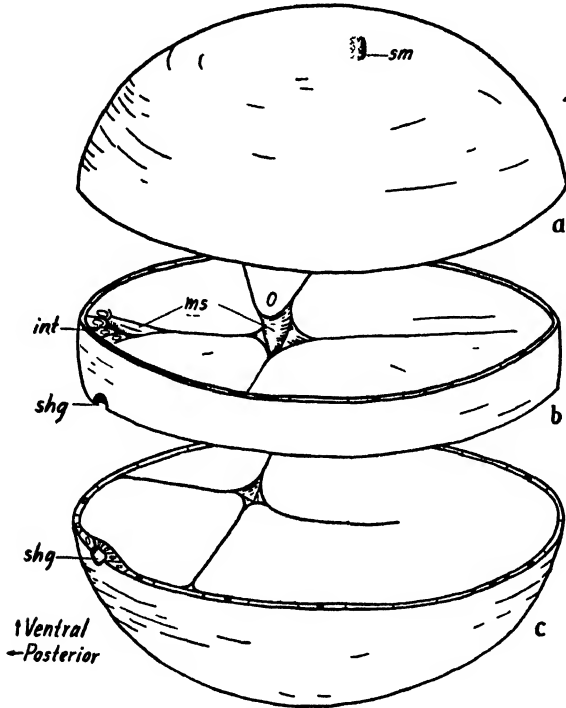


Fig. 1. A larva in the stage of shell-gland invagination, viewed from an oblique angle with the dorsal side below. The larva has been cut frontally and the three sections separated slightly to show internal structures. $\times 200$.

int, intestinal cells; *ms*, mesenteron; *shg*, shell gland; *sm*, stomodaeum.

ment of the various systems, these stages will not be described separately. The name of each stage is given with the accompanying figure. The ambiguous terms veliger and trochophore have been omitted. Terms referring to the adult anatomy are explained in an earlier paper (Moritz, 1938).

DEVELOPMENT OF THE GERM LAYERS

In *Crepidula*, as in most prosobranchs, the ectoderm arises from the first three quartettes. In larva and adult it constitutes the external epithelium and forms the stomodaeum and what little proctodaeum there is.

All entoderm is derived from cells A-D, 4A-4C, and 4d (Conklin's nomenclature; 1897, pp. 153-157). Part of 4d also gives rise to mesoderm, which in turn disperses to form mesoblast cells. Except for the intestinal cells, the ento-

dermal cells at the stage of shell-gland invagination (fig. 1) are large and bear yolk. Entoderm gives rise to all the gut and accessory structures from the oesophagus to the rim of the anus.

Two mesodermal bands are derived from 4d. These disperse as mesoblasts before the stage of shell-gland invagination. Three other cells give rise to mesoblasts (Conklin, 1897, pp. 149–151). These cells are found one each in quadrants A, B, and C, and are of the second quartette. Conklin was unable to find in the fourth quadrant a cell of the second quartette which gave rise to mesoblasts. He believes (1897, p. 151, fn.) that the derivation of mesoblasts indirectly from 4d is substituted for a derivation of the mesoblasts from cells derived from 2d. But he does not deny the possibility that mesoblastic cells may arise from derivatives of 2d at a later time than gastrulation.

During the stage of elongation (fig. 3), mesoblasts seem to arise from the anlagen of the ctenidium and endostyle and from the dorsal region of the shell gland (fig. 17, *eo*). Cells forming the shell gland are derived from 2d (Conklin, 1897, p. 140), and no doubt the cells of the anlagen of the ctenidium and endostyle are also derived from that source. These mesoblasts take part in the formation of the adult excretory organ, pericardium, and heart. They probably represent the mesoblasts of the second quartette that are missing during gastrulation.

As the remaining mesoblasts increase, they migrate to all parts of the cavity between the ectoderm and entoderm and eventually develop into the muscle cells and connective tissue of the adult. In the head vesicle of the larva they form myoblasts (fig. 15, *my*), the unicellular "muscles" of the larva (Lillie, 1895, p. 38).

TORSION

Torsion is a twist of the right side dorsally to the left and of the left side ventrally to the right. In *C. adunca* it proceeds through about 135°, and is a result of dissimilar growth of the two sides of the larva. It is first noticeable in the stage of the foot anlage, where the right and left larval excretory cells begin to move dorsally and ventrally, respectively. It does not cease until hatching occurs. The process of torsion, at first slow, is more rapid from the stage of the tentacular anlagen through the stage of mantle ascension (figs. 4–6; see the position of the supraoesophageal or visceral ganglia in these figures).

Garstang (1928) stated that torsion probably was a result of asymmetrically placed muscles. Patten's work (1886) suggests this and Smith's work (1935) confirms it for *Patella*. It is otherwise in *C. adunca*. Muscle fibers do not develop until torsion is nearly completed. Myoblasts, it is true, are present during torsion. They are, however, irregularly scattered and never aggregated more in one site than in another. Furthermore, no one myoblast, nor even a group of them, attains a size comparable to the muscle fibers in *Patella* which effect torsion. Hence, if torsion of the ancestral form was due to asymmetrically placed muscle fibers, it is only the tendency to asymmetrical growth that has been inherited in *C. adunca*.

DEVELOPMENT OF THE INTEGUMENTARY SYSTEM

ECTODERMAL GLANDS

Anterior pedal gland.—During late mantle descension, the tip of the propodium develops a small invagination. This is the anlage of the anterior pedal gland. Its cells divide rapidly, and at the time of hatching the entire forward end of the propodium is filled with the resulting compound alveolar gland,

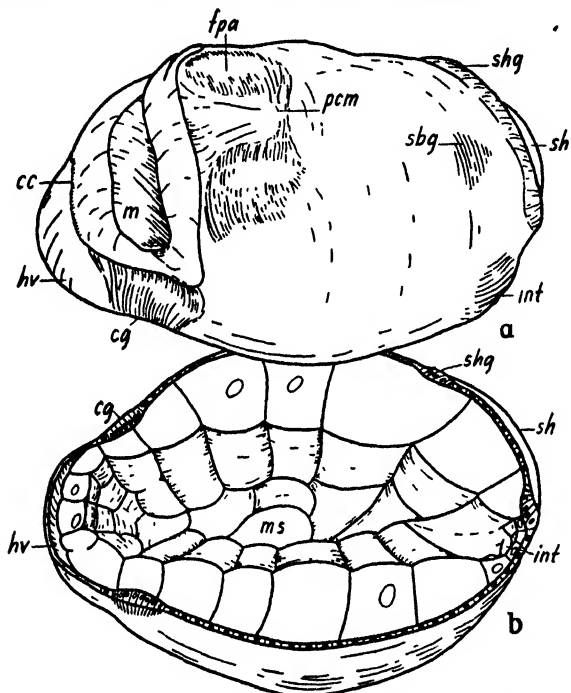


Fig. 2. A larva in the stage of the foot anlage. The larva has been cut in a frontal plane and the two halves separated and viewed obliquely with the dorsal half at the bottom of the figure. $\times 200$.

cc, cerebral commissure; cg, cerebral ganglion; fpa, foot anlage and pedal ganglion anlagen; hv, head vesicle; int, intestine; m, mouth; ms, mesenteron; pcm, pedal commissure; sbg, suboesophageal ganglion; sh, shell; shg, shell gland.

but its maximum growth is not attained until the individual has reached sexual maturity. The point of invagination remains at the anterior edge of the propodium as the opening of the gland to the exterior.

Shell gland (e.g., fig. 4b, shg).—The term shell gland has been used to designate the rim of ectodermal tissue at the periphery of the shell (figs. 2–5, shg). Invagination of the ectoderm at the posterior end of an embryo in the stage when the stomodaeum is being formed is the first indication of the gland (fig. 1, shg). The actual secreting structures are a series of alveolar invaginations along this rim (figs. 4a, 7a, shg). At first unicellular, they enlarge to form compound alveolar glands. When the shelf lamina (fig. 6, sfl) is

developed, the shell-secreting glands are carried posteriorly in its tip. During mantle descension, the ectodermal rim of the mantle, namely, the shell gland, assumes its adult condition as the periphery of the mantle fold from the shelf of the shell forward and as the accessory mantle fold around the posterior half of the animal. The glands lie within the cavity of the ectodermal rim and open by their points of invagination at the shell periphery (fig. 20).

THE FOOT

The first indication of the development of the foot occurs at the time the oral plate ruptures (fig. 2a). Two darkly staining areas in whole mounts may be observed on the ventral surface posterior to the mouth. Origin of the foot,

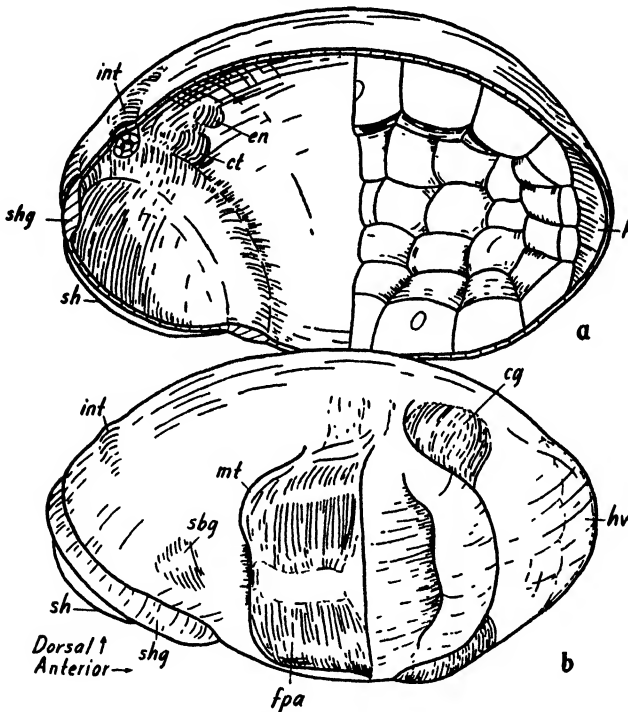


Fig. 3. A larva in the stage of elongation or endostylar anlage. The larva has been cut in a frontal plane and the halves separated and shown tipped to the left. The dorsal half (a) includes the yolk cells. These have been removed from the posterior half (b) so that the underlying structures may be seen. $\times 200$.

cg, cerebral ganglion; ct, ctenidial anlage; en, endostylar anlage; fpa, foot and developing pedal ganglia; hv, head vesicle; int, intestine; mt, metapodium; sbq, suboesophageal ganglion; sh, shell; shg, shell gland.

however, is not bilateral, for what appears to be two lobes is actually one mound consisting of two darkly staining areas separated by an area of large, clear, ciliated cells.

The metapodium differentiates first from the foot anlage (fig. 3b, *mt*). It becomes more pronounced as the larval heart appears (fig. 5b, *mt*). The propodium develops at the same time as the larval heart. The mesopodium is the

residual portion of the foot. During late mantle ascension (fig. 6), the three regions of the foot are well marked. The metapodium has yet to acquire its definite dorsal surface from the shelf lamina (fig. 6, *sfl*), which is beginning its posterior growth. After the shelf lamina has reached its maximum extent, the space between it and the metapodium, the sublamine space (fig. 15, *sbs*), becomes successively shallower. The shelf lamina, with the exception of its periphery, is thus incorporated into the metapodium. The periphery forms the accessory mantle fold (Moritz, 1938, fig. 2), and the sublamine space remains in the adult as the accessory mantle cavity.

BODY CILIATION AND THE VELUM

Cilia cover the surface of the stomodaeum (fig. 13, *sm*), and as the head vesicle (figs. 2-7, 14, 15, *hv*) and clear area of the foot (figs. 2a, 3b) develop, cilia cover them.

Development of the velum begins in the stage of the foot anlage. In contrast to the powerful velum of some prosobranch larvae, for example *Patella* (Patten, 1886), the velum of *C. adunca* is poorly developed. Bands of cilia arise at each corner of the mouth and extend gradually upward and along the posterior edges of the developing cerebral ganglia. Posterior and anterior branches of the velum at its dorsal end do not occur as in *C. fornicata* (Conklin, 1897, p. 136). As the head vesicle grows dorsally to include the area above the cerebral ganglia, the velar cilia and those of the head vesicle become continuous, and thus the entire anterior surface of the larva is ciliated in the stage of the anlagen of the tentacles, with the exception of the anlagen of the cerebral ganglia and the tentacular anlagen. The velar band is four cells wide at maximum development in the stage of the tentacular anlagen (fig. 4a, *ve*). During mantle ascension the postoral ciliary band begins to retrogress. Further diminution occurs during mantle descension, and at the same time the head vesicle begins to collapse. Early young adults bear vestiges of the velum at the posterior margin of the bases of the tentacles. On the right side the velum may contribute to the formation of the ciliary band for sperm transfer; it disappears entirely on the left side. The cells of the head vesicle absorb their cilia and metamorphose into ordinary epithelial cells.

Cilia, which represent the telotroch, are present as an isolated patch in the stage of the anlage of the endostyle, ventral to the anlage of the intestine. As the shell advances, this patch is encroached upon and finally obliterated.

DEVELOPMENT OF THE ALIMENTARY TRACT AND ASSOCIATED ORGANS

Endostyle.—To the right of the middorsal line anterior to the shell gland during elongation (fig. 3, *en*) an irregular mass of cells can be distinguished. Rapid growth of these cells soon pushes a hollow lip outward and downward to the right over the surface of the larva (figs. 18, 4b, 5a, *en*). This lip has a posterior and an anterior part. The posterior half is the anlage of the endostyle; the anterior half, which becomes scalloped, is the developing ctenidium. The process of torsion (cf. figs. 4b, 5a) carries both of these organs toward the left side of the larva, but their growth is so rapid in a diagonally trans-

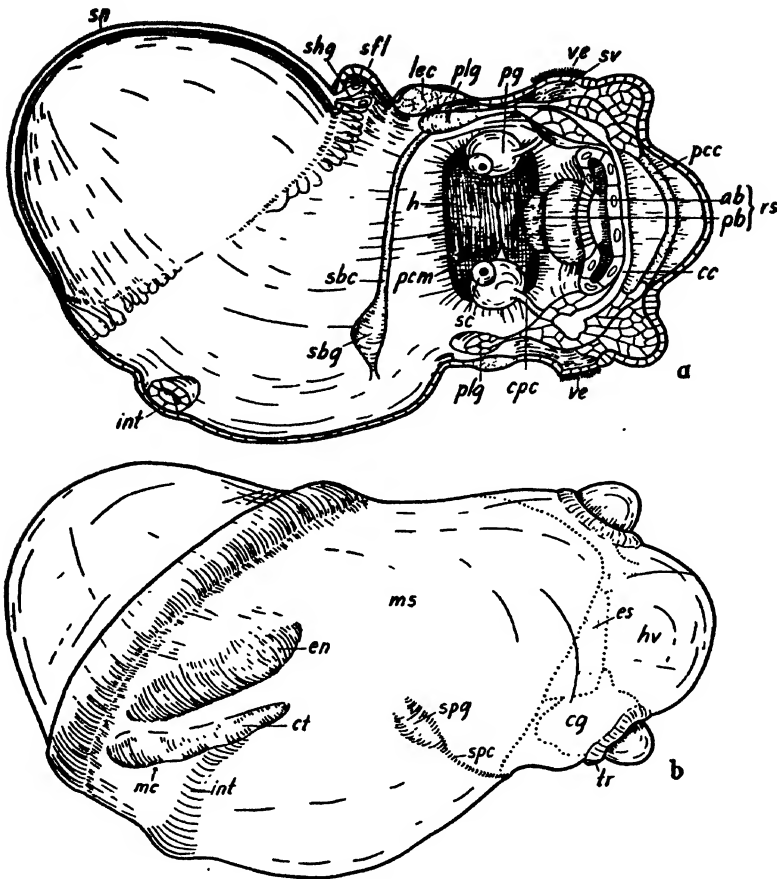


Fig. 4. A larva in the stage of the tentacular anlagen. The larva has been cut in a frontal plane and the halves separated. Both halves are viewed dorsally. From the ventral half (a) the yolk cells have been removed so that other structures may be seen. $\times 200$.

ab, anterior bulb; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpc*, cerebropedal connective; *ct*, ctenidium; *en*, endostyle; *es*, oesophagus; *h*, head vesicle; *hv*, head vesicle; *int*, intestine; *lec*, larval excretory cells; *mc*, mantle cavity anlage; *ms*, mesenteron; *pb*, posterior bulb; *pcc*, pseudocerebral commissure; *pcm*, pedal commissure; *pg*, pedal ganglion; *plg*, pleural ganglion; *rs*, radular sac; *sbc*, suboesophageal connective; *sbq*, suboesophageal ganglion; *sc*, statocyst; *sfl*, shelf lamina; *shg*, shell gland; *spc*, supraoesophageal connective; *spg*, supraoesophageal ganglion; *sv*, subvelar space; *tr*, tentacular ring; *ve*, velum.

verse direction that their position is eventually not wholly on the left side, but also dorsal (fig. 6, *ct, en*). The endostyle, at first posterior to the ctenidium, gradually becomes dorsal during mantle ascension (fig. 6, *en*), then ventral during mantle descension (fig. 7a, *en*), and maintains this position

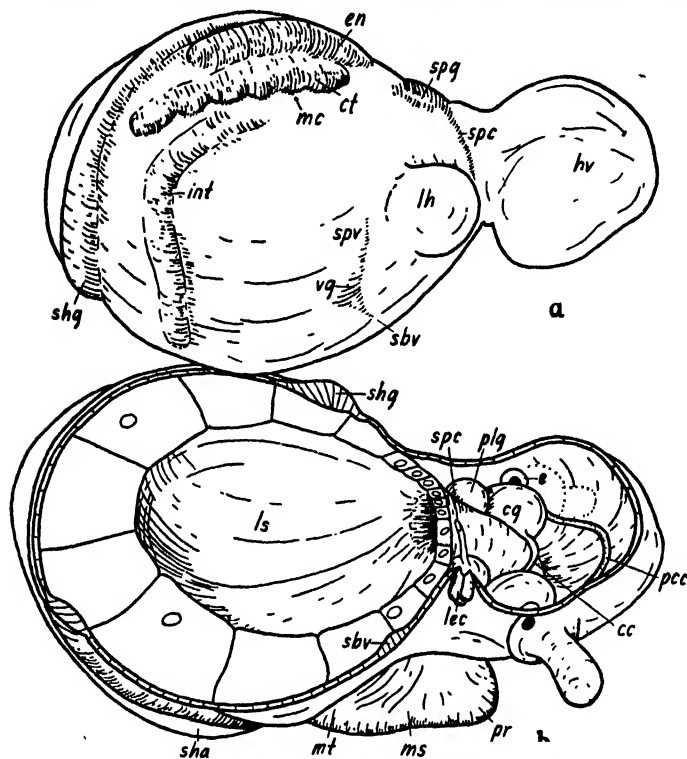


Fig. 5. A larva in the stage of the appearance of the larval heart. The larva has been cut in a frontal plane. The halves are separated and seen from an oblique dorsal view. $\times 200$.

cc, cerebral commissure; *cg*, cerebral ganglion; *ct*, ctenidium; *e*, eye; *en*, endostyle; *hv*, head vesicle; *int*, intestine; *lec*, larval excretory cells; *lh*, larval heart; *ls*, larval stomach; *mc*, mantle cavity anlage; *ms*, mesopodium; *mt*, metapodium; *pcc*, pseudo-cerebral commissure; *plg*, pleural ganglion; *pr*, propodium; *sbc*, subesophageal-visceral connective; *sha*, shell apex; *shg*, shell gland; *spc*, supraesophageal connective; *spg*, supraesophageal ganglion; *spv*, supraesophageal-visceral connective; *vg*, visceral ganglion.

relative to the ctenidium in the adult. The characteristic columnar secretive cells of the endostyle (fig. 20, *en*) appear toward the close of mantle descension.

Ciliated food groove of the right lappet.—Development of this groove (Moritz, 1938, fig. 3, *fg*) is accomplished by invagination of epithelial cells on the dorsal surface of the right lappet of the neck, which develops after hatching. This groove extends from the base of the neck forward to a short distance from the right corner of the mouth.

Food pouch.—On the anterior wall of the mantle cavity and to the right of the mid-line (Moritz, 1938, fig. 3, *fp*) the mantle forms a broad, shallow groove

shortly after the young adult stage. Two broad lips evaginate from the mantle epithelium to form this groove, which is the food pouch.

Stomodaeum, mouth.—The stomodaeum develops at the site of closure of the blastopore. The oral plate (fig. 13, *sm*) ruptures at about the time the foot anlage is first distinguishable, forming the mouth (cf. figs. 13, 14).

Buccal cavity.—The adult buccal cavity is that portion of the alimentary tract which is included in the buccal mass. The posterior part of this cavity is

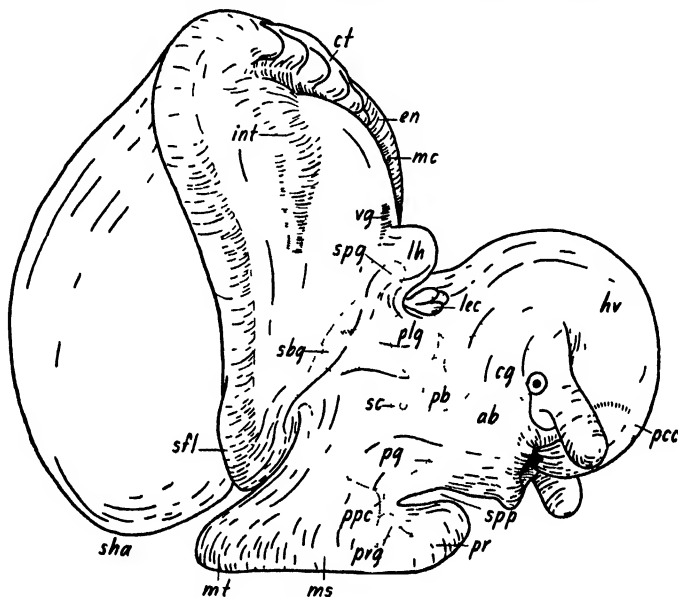


Fig. 6. A larva in the stage of mantle ascension. $\times 200$.

ab, anterior bulb; *cg*, cerebral ganglion; *ct*, ctenidium; *en*, endostyle; *hv*, head vesicle; *int*, intestine; *lec*, larval excretory cells; *lh*, larval heart; *mc*, mantle cavity; *ms*, mesopodium; *mt*, metapodium; *pb*, posterior bulb; *pcc*, pseudocerebral commissure; *pg*, pedal ganglion; *plg*, pleural ganglion; *ppc*, pedal-propodial connective; *pr*, propodium; *prg*, propodial ganglion; *sbg*, subesophageal ganglion; *sc*, statocyst; *sfl*, shelf lamina; *sha*, shell apex; *spg*, supraesophageal ganglion; *spp*, supra-propodial pocket; *vg*, visceral ganglion.

the pharynx, which lies immediately above the orifice of the radular sac. The buccal cavity and its derivatives are ectodermal; entoderm begins with the oesophagus. After the larva is hatched, two jaws are secreted in the dorsal wall of the buccal cavity. The epithelium invaginates to form two sacs, each sac secreting at its base a chitinous tooth.

Development of the radular sac begins soon after the rupture of the oral plate. On the posterior surface of the buccal cavity (fig. 14, *rad*) a plate of nonciliated cells stains more deeply with Delafield's hematoxylin than do the surrounding cells. During elongation the center of the plate invaginates posteriorly to form the posterior bulb of the radular sac (fig. 4a, *pb*). The peripheral region of the plate, which does not invaginate until the tentacles appear, forms the anterior bulb (fig. 4a, *ab*). In sagittal sections of a larva in the stage of the tentacular anlagen, the beginning of a sublingual pocket can be seen

at the junction of the anterior bulb with the lower lip. This pocket marks off a small hump which is the tongue (fig. 15, *to*). At mid-torsion the cells of the posterior and ventral wall of the posterior bulb have changed into odontoblasts (Rössler, 1885, p. 454). These cells secrete the basal plate of the radula. The cells on the dorsal surface, called the dorsal epithelium of the radula by Bloch (1896, pp. 365, 391), become tonguelike and secrete the teeth of the radula (fig. 15). As the basal plate is formed, it is moved forward and upward over the surface of the tongue. The cells of the dorsal epithelium of the radula hang pendant onto the surface of the basal plate and secrete the teeth of the radula, which fuse to the plate and are also carried forward. Bloch (1896) has given an account of the development of the radula in *Viviparus* with which *Crepidula* seems to agree.

Mesenteron.—Mesenteron is the term given to all the gut derived from the entodermal cells. During the stage of shell-gland invagination, it includes two separate cavities (fig. 1b, *ms*): (1) the cavity surrounded by the large yolk cells, that is, the larval stomach, and (2) the cavity formed between the small intestinal cells and their contiguous large yolk cells. After the oral plate ruptures, the posterior yolk cells divide and move laterally so that a continuous mesenteronic cavity is formed, uniting mouth with intestine (fig. 2b, *ms*). The rudiments of the oesophagus and intestine are thus established, with a large cavity connecting the two (fig. 14, *ls*). This middle cavity may be called the larval stomach, since it is the storage cavity of the larva. From the mesenteron arise the oesophagus, the stomach and its accessory parts, and the intestine.

Oesophagus.—The anterior end of the oesophagus marks the junction of ectoderm with entoderm. The oesophagus is lined with cilia, and this distinguishes it from the nonciliated gastric cavity (fig. 12, *es*, *st*). The entrance of the oesophagus into the larval stomach is sufficiently dorsal in a pre-torsional stage to be carried slightly to the left during torsion. Toward the end of mantle descension the oesophagus moves to the left and then inward toward the mid-line. In this way it "cleaves" the anterior diverticulum of the digestive gland from the posterior (cf. figs. 7b, 10, *es*) so that in a young adult the oesophagus (figs. 8–10) passes sharply upward between the posterior and anterior lobes of the digestive gland to enter the posteroventral floor of the stomach.

Stomach, crystalline style, sac, and gastric shield.—As mantle ascension proceeds, the dorsal wall of the larval stomach is rolled forward over the point of junction of oesophagus and larval stomach (figs. 6, 7). Transverse constriction of the larval stomach occurs during late mantle ascension on the ventral wall, and on the right and left lateral walls, and to a lesser degree on the dorsal wall (fig. 7), dividing the larval stomach (fig. 7, *ac*, *pc*) into an anterior cavity and a posterior cavity.

Derivatives of the posterior cavity are the posterior diverticulum of the digestive gland and the greater part of the adult stomach (fig. 12, *pd*, *st*). In sagittal sections of young adults this posterior cavity is clearly marked into two regions (fig. 12, *st*, *pd*). The ventral region is the future posterior diver-

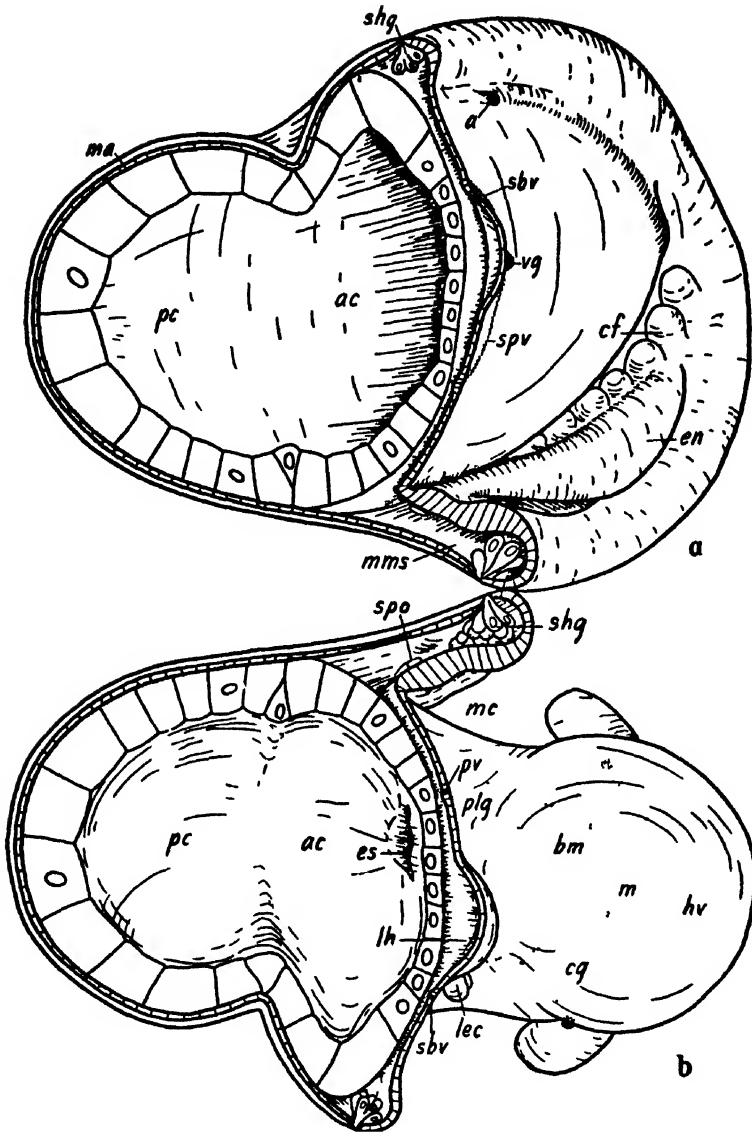


Fig. 7. A larva in the stage of mantle descension. The larva has been cut in a frontal plane and the dorsal half, at the top of the figure, turned with the ventral face upward. $\times 200$.

a, anus; ac, anterior cavity; bm, buccal mass; cf, ctenidial filament; cg, cerebral ganglion; en, endostyle; es, oesophagus; hv, head vesicle; lec, larval excretory cells; lh, larval heart; m, mouth; ma, mantle; mc, mantle cavity; mms, marginal mantle sinus anlage; pc, posterior cavity; plg, pleural ganglion; sbv, suboesophageal-visceral connective; shg, shell gland; spo, supraoesophageal-osphradial connective; spv, supraoesophageal-visceral connective; vg, visceral ganglion.

ticulum of the digestive gland. The dorsal region is the future adult stomach. The cells of the dorsal region secrete a cuticle (fig. 12, *c*) during the young adult stage, and on the posterior wall produce a bladeliike ridge, the chitinous gastric shield (fig. 11, *gs*).

Derivatives of the anterior cavity (fig. 12, *ac*) are the anterior diverticulum

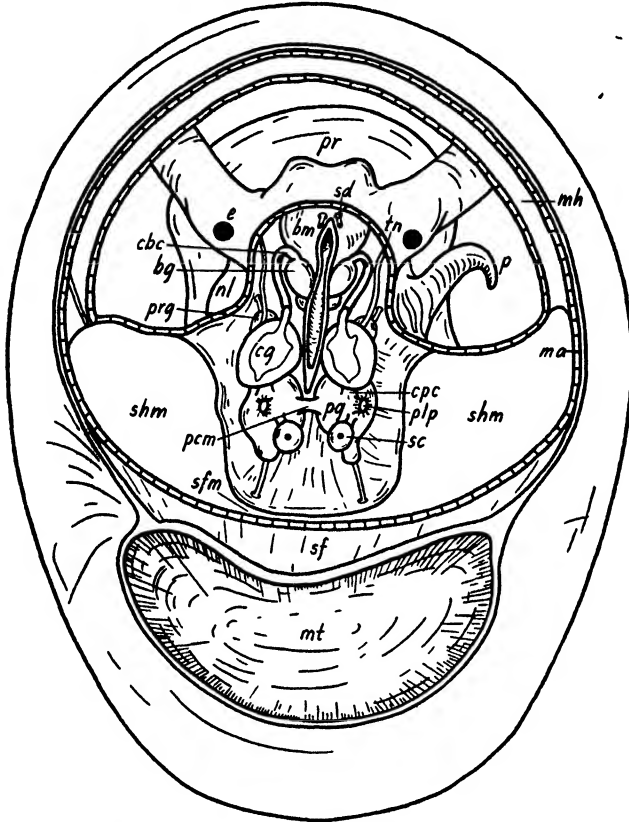


Fig. 8. Figures 8, 9, 10, and 11 illustrate a young adult cut in four successive layers in the frontal plane. Figure 8 has the dorsal surface turned upward. Figures 9, 10, and 11 have the ventral face turned upward. $\times 200$.

bg, buccal ganglion; *bm*, buccal mass; *cbc*, cerebrobuccal connective; *cg*, cerebral ganglion; *cpc*, cerebropedal connective; *e*, eye; *ma*, mantle; *mh*, mantle hemocoel; *mt*, metapodium; *nl*, neck lappet; *p*, penis; *pcm*, pedal commissure; *pg*, pedal ganglion; *plp*, pleuropedal connective; *pr*, propodium; *prg*, propodial ganglion; *sc*, statocyst; *sd*, salivary duct; *sf*, shell shelf; *sfm*, shelf muscle; *shm*, shell muscle; *tn*, tentacular nerve.

of the digestive gland, a portion of the adult stomach, the style sac, and the gastric end of the intestine. A shift in position of the gastric end of the oesophagus is concerned with the formation of these derivatives. During early mantle descension, the oesophagus enters the larval stomach anteriorly and slightly to the left (fig. 7b, *es*). As mantle descension enters its final phase, the oesophagus, possibly through the effect of torsion, swings to the left and then inward toward the mid-line. Simultaneously a sagittal division of the

anterior cavity of the larval stomach occurs; this produces right and left chambers. The right chamber concurrently extends forward, to the left, and under the left chamber, folding the oesophagus between the anterior and posterior diverticula of the digestive gland (fig. 10, *es*). It is the right chamber which forms the anterior diverticulum of the digestive gland. The left cham-

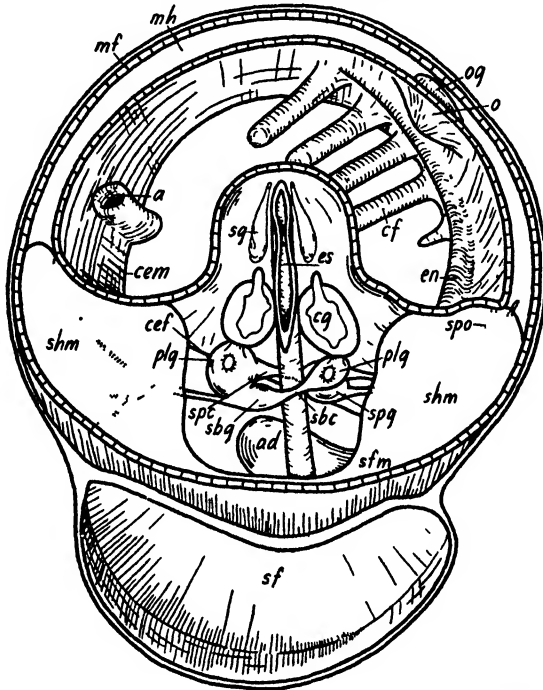


Fig. 9. A frontal section of a young adult taken above the section shown in figure 8. The ventral face is turned upward. $\times 200$.

a, anus; *ad*, anterior diverticulum of the digestive gland; *cef*, cut edge of the floor of the mantle cavity; *cem*, cut edge of the mantle; *cf*, ctenidial filament; *cg*, cerebral ganglion; *en*, endostyle; *es*, oesophagus; *og*, osphradium; *og*, osphradial ganglion; *plg*, pleural ganglion; *sbc*, suboesophageal connective; *sbg*, suboesophageal ganglion; *sf*, shell shelf; *sfm*, shelf muscle; *sg*, salivary gland; *shm*, shell muscle; *spc*, supraoesophageal connective; *spg*, supraoesophageal ganglion; *spo*, supraoesophageal-osphradial connective.

ber develops into the style sac and gastric end of the intestine, divided from each other by a fold which arises after the larva hatches.

Intestine.—The establishment of the intestine has already been considered. Its subsequent growth and position to the time of mantle descension are shown in figures 3 to 6. Then, during mantle descension, the mantle cavity recedes, the ctenidium and endostyle roll onto its dorsal surface (cf. figs. 6, 7a, *en*), and the intestine is caught between the walls of the mantle fold (cf. figs. 7a, 10), where it is folded into an S shape as viewed dorsally in a young adult (figs. 9, 10, and Moritz, 1938, fig. 3). The right or upper termination of the

S is the anus, the left or lower termination is the junction with the stomach. The anterior loop (Moritz, 1938, fig. 3, *al*) may be designated as the anal loop, the posterior loop may be called the renal loop, because the excretory organ lies in its curve.

Two changes occur after hatching: (1) The stomach in its shift to the mid-line carries the junction of the intestine with it and formation of a third intestinal loop, the gastric loop (Moritz, 1938, fig. 3, *gl*), occurs. This loop lies below the floor of the mantle cavity. The renal and anal loops lie within the

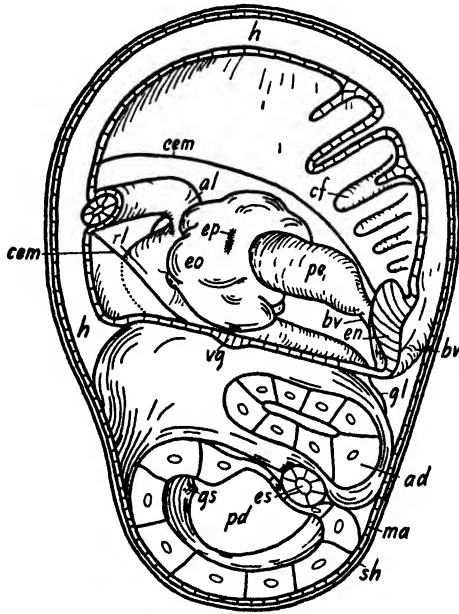


Fig. 10. A frontal section of a young adult taken above the section shown in figure 9. The ventral face is turned upward. $\times 200$.

ad, anterior diverticulum; *al*, anal loop; *bv*, branchial vein; *cem*, cut edge of the mantle; *cf*, ctenidial filament; *en*, endostyle; *eo*, excretory organ; *ep*, excretory pore; *es*, oesophagus; *gl*, gastric loop; *gs*, gastric shield; *h*, hemocoele; *ma*, mantle; *pd*, posterior diverticulum; *pe*, pericardium; *rl*, renal loop; *sh*, shell; *vg*, visceral ganglion.

hemocoele of the mantle fold. (2) The anal loop of the intestine grows extensively to the left and encloses the excretory organ on three of its four sides (Moritz, 1938, fig. 3, *al*, *eo*).

Proctodaeum, anus.—During late mantle ascension, the blind end of the intestine lies in contact with the external epithelium midway up the right side of the front face of the visceral mass (fig. 6, *int*). This place of meeting of ectoderm with entoderm is the anal plate. Invagination of the ectoderm is almost negligible, hence there is little or no proctodaeum. The anal plate ruptures during early mantle descension to form the anus (fig. 7a, *a*).

Salivary glands.—These organs appear in the course of mantle descension.

The anterior wall of each side of the anterior bulb of the buccal mass evaginates. By the time the young adult stage is reached, the glands, formerly lying anterior to the buccal ganglia, have grown dorsally and posteriorly over the ganglia (fig. 9, *sg*). The salivary ducts connect with the buccal cavity above the tip of the tongue (fig. 8, *sd*).

Digestive gland.—Two diverticula compose this organ in *C. adunca*. Their development has been discussed with the development of the stomach.

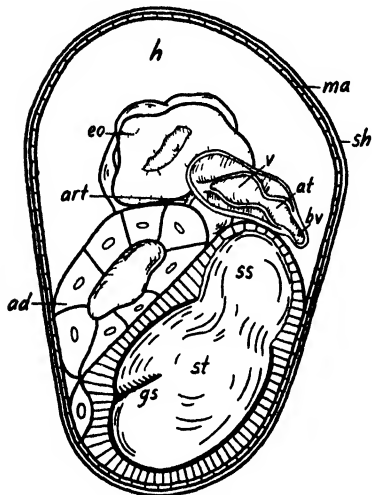


Fig. 11. A frontal section of a young adult taken above the section shown in figure 10. The ventral face is turned upward. $\times 200$.

ad, anterior diverticulum; *art*, arterial trunk; *at*, atrium; *bv*, branchial vein; *eo*, excretory organ; *gs*, gastric shield; *h*, hemo-coele; *ma*, mantle; *sh*, shell; *ss*, style sac and gastric end of the intestine; *st*, stomach; *v*, ventricle.

DEVELOPMENT OF THE NERVOUS SYSTEM

All cells of the nervous system are derivatives of the cells of the first three quartettes and, before the outgrowth of processes, are indistinguishable from the cells of the external epithelium except by position.

DEVELOPMENT OF THE GANGLIA

Formation of all ganglia in *C. adunca* is the same with the exception of the pleural ganglia, which will be discussed shortly. Other ganglia form as follows. Ectodermal cells in the region of the formative ganglion become crowded and columnar, owing to rapid cell division. Shortly after this has occurred, a cross section of the ectoderm forming the ganglion will show that the one-celled layer has increased to two cells in thickness. By continued division of these cells and their derivatives a mound is produced on the internal surface of the external epithelium. This

mound pinches off from the layer from which it is derived and becomes roughly ovoid to form a ganglion. Heath (1916) states that cells arising from the ectoderm migrate inward to form the various ganglia without further cell division. Such migration certainly occurs, but the formation of a ganglion seems to be more than a mere delamination of a cell mass.

Cell division is another matter. It is to be admitted that cell divisions are not readily recognizable among the cells forming the ganglia. Nuclei are small, the chromosome number is large; hence the nucleus of a dividing cell gives the appearance of an overstained resting nucleus. In reality, the dark spots frequently present in ganglia are dividing cells.

A description of the development of only certain of the ganglia is important, since the others offer no unexpected features. The ganglia to be discussed are the pleural ganglia, because they depart from the general developmental method given above; the propedal ganglia, because they have not hitherto

been reported; and the buccal ganglia, because they involve a disagreement with previous reports.

Pleural ganglia.—From the evidence available it is probable that the pleural ganglia do not form as do other ganglia by mass separation of cells from the external epithelium. Rather do the cells of the pleural ganglia straggle in ones, twos, or threes into the hemocoele and there form each ganglion by aggregating.

Cells which later form the pleural ganglia can be observed streaking inward singly from the ectoderm about the time the endostyle forms. Contem-

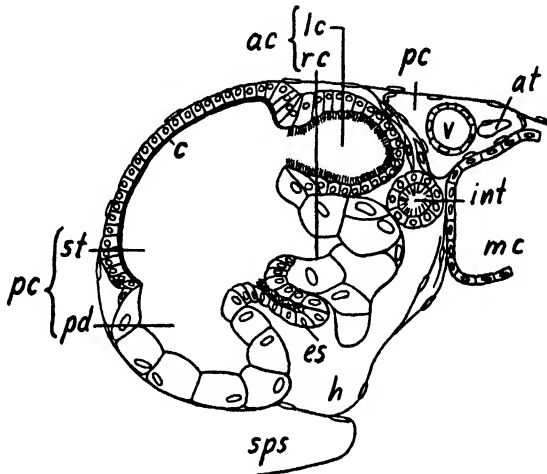


Fig. 12. A parasagittal section of the visceral hump taken to the left of the meson in a young adult, showing the relationship of the organs derived from the larval stomach. $\times 300$.

ac, anterior cavity; at, atrium; c, cuticle; es, oesophagus; h, hemocoele; int, intestine; lc, left chamber; mc, mantle cavity; pc, posterior cavity; pd, posterior diverticulum; rc, right chamber (anterior diverticulum); sps, supralaminar space; st, stomach; v, ventricle.

porary with the appearance of the tentacles, the pleural ganglia begin to take definite ovoid shape (fig. 4a, *plg*). When the larval heart appears, the pleural ganglia (fig. 5b, *plg*) no longer touch the external epithelium. Torsion then produces a slight difference in the levels of the ganglia. The right is pulled upward, the left lowered, and this relationship is permanent. During mantle descension, the suboesophageal ganglion approaches the right pleural, and by the time hatching occurs the two connect (fig. 9, *sbg*, *plg*). This is the adult condition.

Propodial ganglia.—Ventral to the pedal ganglia at the time that the tentacles appear, two masses of cells are budded inward from the epithelium of the foot to form the propodial ganglia (fig. 6, *prg*). By the time the larval heart appears, each propodial ganglion is well defined as a ball appended to the ventral tip of a pedal ganglion. During torsion, fibers develop in these ganglia, and in young adults they are well-defined enlargements on the nerves

running from the pedal ganglia into the propodium (fig. 8, *prg*). In the adult individual they lie close to the anterior and ventral surfaces of the pedal ganglia.

Buccal ganglia.—Rudiments of the buccal ganglia develop during the stage of the appearance of the larval heart (fig. 4, *ab*, *pb*). Cells forming the buccal ganglia arise laterally between the two bulbs of the buccal mass and probably arise from the epithelium which forms the posterolateral walls of the anterior bulb. Heath (1916, p. 484) states that the buccal ganglia "are the only ganglia which do not directly arise from cells migrating from the overlying ectoderm; on the other hand, they give clear evidence of being products of the cerebral ganglia." The slides examined do not offer evidence for that view. It is true that the buccal mass is so compact as to make definite determination of the origin of the buccal ganglia impossible by the technique used. There is evidence, however, to support the opinion that the ganglia arise directly from the ectoderm of the buccal mass. A bridge of cells between the buccal and cerebral ganglia would indicate that the latter had budded off the former; such bridges do not develop until after the cells of the buccal ganglia appear. Furthermore, Smith (1935, p. 119) reports a derivation of the buccal ganglia from the epithelium of the buccal mass in *Patella*.

DEVELOPMENT OF THE COMMISSURES AND CONNECTIVES

Commissures and connectives, as illustrated by the cerebral commissure (figs. 2a, 4a, 5b, 14, 15, *cc*), are derived from the external epithelium lying between the ganglia which each connects. As the ganglia sink inward from the ectoderm and sever themselves from it, a chain of cells on the surface of the larva likewise sinks inward, each end of the chain being continuous with the cells of one of the ganglia. This is not in agreement with Conklin, who states that outgrowths of the cerebral ganglia form the cerebral commissure. It is significant, however, that all commissures and connectives in their early stages show nerve-cell nuclei, suggesting that the nerve trunks arose from a chain of ordinary ectodermal cells. Later in development, each commissure or connective loses the nuclei of the nerve cells and is composed only of their fibers, the nuclei probably having migrated to the ganglia. Moreover, actual protoplasmic bridges are to be observed connecting cells of the ganglia with cells which still form ectodermal epithelium but which will sink inward to form the commissure or connective. Later in development, nerve-cell processes may grow out from cells of a ganglion into the commissure or connective. The initial formation, however, is from cells sunk inward from the ectodermal epithelium.

Posterior to the earliest position of the true cerebral commissure a second chain of cells, the pseudocerebral commissure (fig. 4a, *pcc*), reaching from the right to the left cerebral ganglion, develops during the stage of the tentacular anlagen. This line of cells is clearly evident in whole mounts (fig. 6, *pcc*) and can readily be found in sections (fig. 15, *pcc*). It never sinks inward, but remains until late in the period of mantle descension. Its larval function and fate are unknown. Possibly it represents the connection between the cerebral

commissure and the apical organ described by Conklin (1897, p. 111) for *C. fornicata*. However, *C. adunca* does not possess an apical organ.

DEVELOPMENT OF THE SENSE ORGANS

Eyes.—Simultaneously with the development of the foot anlage, the eyes invaginate from the external epithelium at the posterior border of each cerebral ganglion. Forward extension of the head carries the eyes anterior to the cerebral ganglia to a position beneath the surface of the base of each tentacle (fig. 8, e).

Statocysts.—Invagination of the statocysts occurs soon after the anlage of the foot appears. Their positions are at the posterolateral corners of the foot

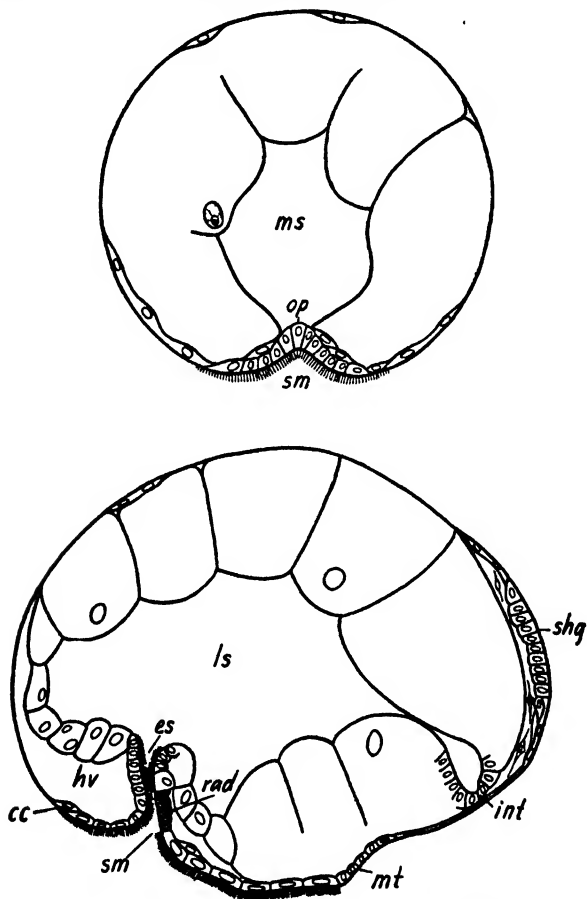


Fig. 13. Cross section through the stomodaeum of a larva in the stage of shell-gland invagination. Yolk is not indicated in the large cells. $\times 225$.

Fig. 14. Sagittal section of a larva in the stage of the foot anlage. Yolk is not indicated in the large cells. $\times 225$.
cc, cerebral commissure; es, oesophagus; hv, head vesicle; int, intestine; ls, larval stomach; ms, mesenteron (equivalent to all three organs, the oesophagus and the larval stomach and the intestine); mt, metapodial anlage; op, oral plate; rad, radular sac anlage; shg, right edge of the shell gland; sm, stomodaeum.

anlage. As the pedal ganglia develop, each statocyst is carried upward on one of the ganglia. The final position of each statocyst is above the posterior tip of a pedal ganglion and slightly toward the mid-line of the larva (fig. 4a, sc). A single statolith is secreted in each vesicle in the stage of the tentacular anlagen.

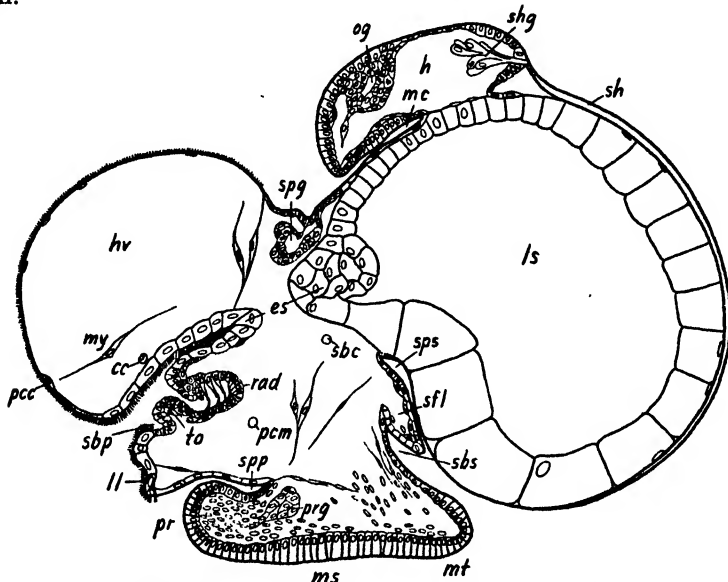


Fig. 15. Sagittal section through a larva in the stage of mantle ascension. $\times 225$.

cc, cerebral commissure; es, oesophagus; h, hemocoel; hv, head vesicle; ll, lower lip; ls, larval stomach; mc, mantle cavity; ms, mesopodium; mt, metapodium; my, myocyte; og, osphradial ganglion; pcc, pseudocerebral commissure; pcm, pedal commissure; pr, propodium; prg, propodial ganglion; rad, radular sac and radula; sbc, suboesophageal connective; sbp, sublingual pocket; sbs, sublaminate space; sfl, shelf lamina; sh, shell; shg, shell gland; spg, supraoesophageal ganglion; spp, suprapropodial pocket; sps, supralaminar space; to, tongue.

Tentacles.—The tentacles first appear as mounds in the lateral areas of the head vesicle above the corners of the mouth (fig. 4). From the beginning they are filled with mesoblast cells. During the earlier part of tentacular development, each tentacle is encircled at its base by a ring, the tentacular ring (fig. 4b, tr), which persists to, and sometimes well into, the period of mantle ascension. By the time the anus is formed the tentacular rings are undistinguishable.

Osphradium.—Appearance of the osphradium does not occur until the spat are ready to hatch. At that time a swelling appears in the ectodermal epithelium ventral to the anterior half of the endostyle. This swelling lying over the osphradial ganglion is the osphradial anlage (fig. 9, o). After hatching occurs, the swelling becomes undulated into from six to eight hillocks, and by the time the male is 3 or 4 mm. long, from six to eight blunt projections are present (Moritz, 1938, fig. 4, o). Projections lie only on one side of the axis, quite unlike the condition described by Kleinstüber (1913) for *Calyptraea*.

DEVELOPMENT OF THE EXCRETORY SYSTEM

Larval system.—Larval excretory cells are present in *Crepidula adunca*, derived from the second or third quartette cells. Conklin (1897, p. 144) states that in *C. fornicata* they may arise from cells $3c^{11}$ and $3d^{11}$, but he takes his evidence from the work of Heymons (1893) on *Umbrella*.

Anlagen of these cells appear in *Crepidula* posterior and dorsal to the posterior end of the foot. The stomodaeum is in the process of formation at that time. When the oral plate ruptures to form the mouth, these future larval

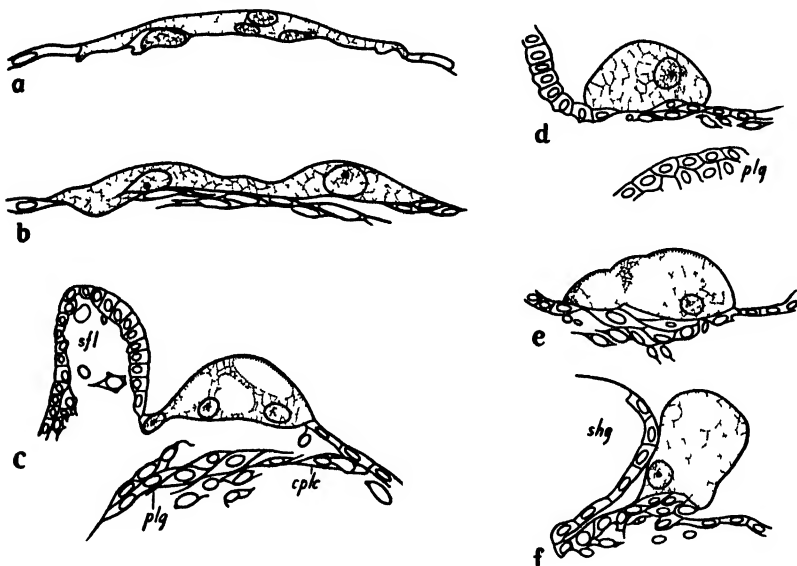


Fig. 16. Development of the left larval excretory cells. All are drawn in frontal view. $\times 400$.

cplc, cerebropleural connective; *plg*, pleural ganglion; *sfl*, shelf lamina; *shg*, shell gland or mantle edge.

excretory cells metamorphose from the ordinary ectodermal type to large, clear, vacuolated cells (fig. 16a). They soon bulge prominently from the surface (figs. 16b, 4a, *lec*). Shortly after the anlagen of the tentacles are present, ectoderm begins to push beneath the cells (fig. 16d). This continues until a complete layer has been formed (fig. 16e). The excretory cells are then "rolled" from the surface, on the left side by the advancing mantle edge (fig. 16f), on the right side by the pulsating larval heart. They have no part in the formation of the adult excretory organ; Kleinstaub's interpretation (1913, p. 438) of Conklin's work is in error on this point.

Adult system.—An accumulation of mesoblasts in the region of the dorsal lip of the shell gland during the stage of elongation (fig. 17, *eo*) is the anlage of the adult excretory organ and pericardium. These mesoblasts are probably derived from the shell gland and the combined anlagen of the ctenidium and endostyle. Hence the excretory organ and pericardium probably are composed of some of the derivatives of 2d. This supports Meissenheimer's conten-

tion (1898, 1901a, 1901b) that these organs are ectodermal in origin. It is possible, but it does not seem probable, that mesoblasts from 4d also contribute to this mass of cells.

Formation of the mantle cavity begins shortly after the anlage of the adult excretory organ appears. The mantle cavity begins as a slight groove in front

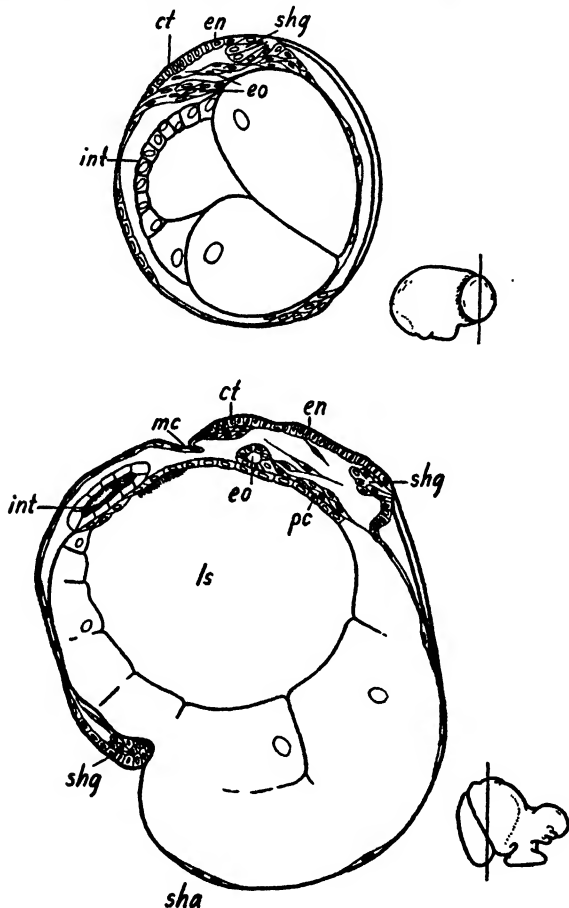


Fig. 17. Transverse section through a larva in the stage of elongation. $\times 225$.

Fig. 18. Transverse section through a larva in the stage of the appearance of the larval heart. $\times 225$.

ct, ctenidial anlage; en, endostylar anlage; eo, excretory organ anlage; int, intestinal cells; ls, larval stomach; mc, mantle cavity anlage; pc, pericardial anlage; sha, shell apex; shg, shell gland.

of the anlage of the ctenidium (figs. 18, 5a, mc). The bottom of this groove is in contact at one point with the block of mesoblastic cells beneath. At this point a short cord of tissue connects the epithelium of the mantle cavity with the block. At first solid, as is the block, the cord acquires a lumen during the shift of the supraesophageal ganglion from right to left. Simultaneously, the

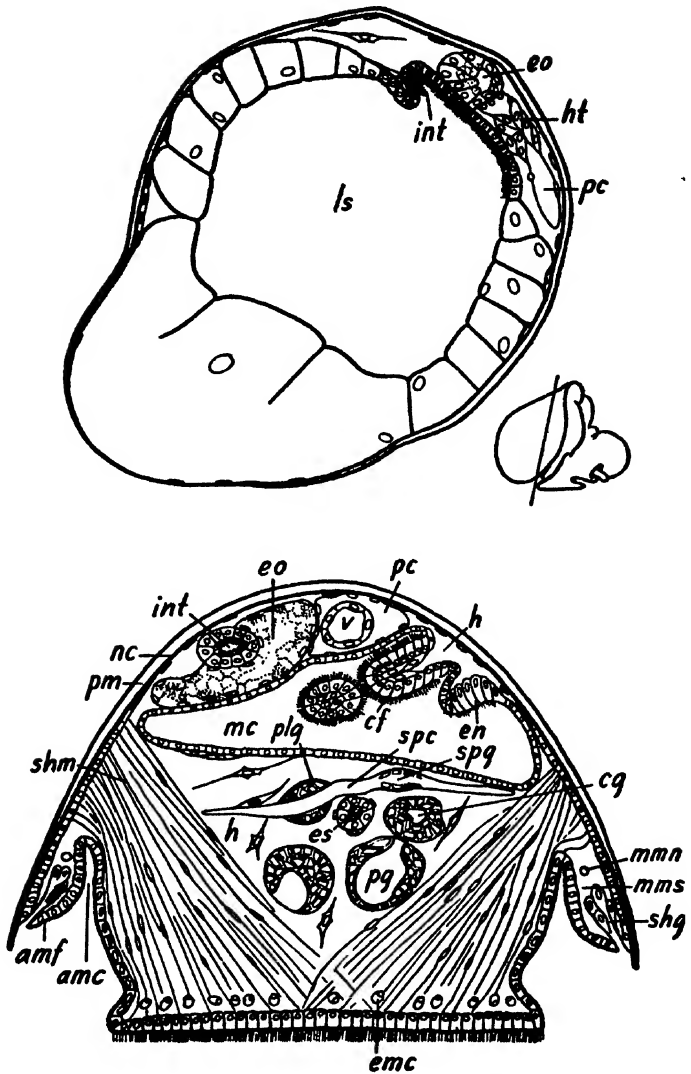


Fig. 19. Transverse section through a larva at the end of mantle ascension and at the beginning of mantle descension. $\times 225$.

Fig. 20. Transverse section through a young adult. $\times 225$.

amc, accessory mantle cavity; *amf*, accessory mantle fold; *cf*, ctenidial filament; *cg*, cerebral ganglion; *emc*, early muscle cell; *en*, endostyle; *eo*, excretory organ; *es*, oesophagus; *h*, hemocoel; *ht*, heart anlage; *int*, intestine; *ls*, larval stomach; *mc*, mantle cavity; *mmn*, marginal mantle nerve; *mms*, marginal mantle sinus; *nc*, nacreous layer; *pc*, pericardial cavity; *pg*, pedal ganglion; *ppl*, pleural ganglion; *pm*, periostracum; *shg*, shell gland; *shm*, shell muscle; *spc*, supraesophageal connective; *spg*, supraesophageal ganglion; *v*, ventricle.

center of the block becomes successively lighter in stained sections, nuclei move toward the periphery followed by the cytoplasm, and a lumen appears (figs. 18, 19, *eo*). The lumen connects with the short duct which opens into the mantle cavity.

Growth of the mantle fold forward and downward carries the excretory organ forward in its hemocoel (figs. 10, 11, *eo*). Increase in the size of the lumen is not marked until after hatching. In the young adult stage the organ consists of a loose network of clear cells (fig. 20, *eo*) surrounding a small cavity. These cells closely resemble reticulate connective tissue. After hatching occurs, the excretory organ increases its growth rapidly to the right, pushing ventral to the intestine. Infolding then occurs to enlarge the functional surface of the excretory organ, which opens into the mantle cavity by a ciliated slit (fig. 10, *ep*) formed from the short duct. The slit lies just above the brain region.

A renopericardial pore was not found at any stage of development, and there is no development comparable to the description given by Miss Drummond for *Viviparus (Paludina)* (1902, pp. 99–102), confirming the work of Erlanger (1891), that the pericardium buds off the excretory organ, the renopericardial pore remaining as the minute connection between the two cavities. Kleinstüber (1913, p. 437) states that all Monotocardia possess this pore, a thesis with which many writers on the subject are in agreement and which is true for many prosobranchs studied so far. It is probable, therefore, that *C. adunca* does possess the pore, but the situation presented by the cells in the vicinity of the pericardium is so confusing as to make it unwise to venture a definite statement.

DEVELOPMENT OF THE RESPIRATORY SYSTEM

Head vesicle.—This organ is probably the larval organ of respiration, since its exposed thin membrane (fig. 6, *hv*) may easily allow the exchange of gases between the hemocoelic fluid and the exterior. Its first appearance is in the stage of the foot anlage (figs. 2, 14, *hv*) as a clear space in the anteroventral region of the forward end. Gradual enlargement occurs (fig. 3, *hv*) until it forms half of the preoral region of an elongating larva. It is globular in form (figs. 4, 5, *hv*) during early mantle ascension. By the end of mantle ascension the head vesicle has reached its maximum growth (fig. 6, *hv*). Ciliated over its entire surface, it is composed of large, thin, clear cells through which the internal organs are clearly visible (fig. 7b). As mantle descension occurs, the head vesicle diminishes in size until it forms a somewhat wrinkled mass of tissue in the head region. These cells probably form the dorsal epithelium of the head and neck.

Ctenidium.—Anterior to the ridge of cells forming the anlage of the endostyle (fig. 4b, *en*) is a second ridge, the anlage of the ctenidium. Bothanlagen arise in an elongating larva (fig. 3a, *ct, en*) from a common mass of cells to the right of the middorsal line and in front of the shell gland. Soon after the two ridges become distinguishable, the anterior one appears scalloped because of

the development of about four slight mounds on its anteroventral surface (fig. 4a, *ct*). These scallops are the rudiments of the ctenidial filaments. Additional rudiments are added (figs. 5a, 6, 7a, 9, 10, *cf* or *ct*) until nine or more are present in the young adult stage. The scallops, during late mantle ascension, push outward to form small nipples, and these, from descension to hatching, become fingerlike. Cilia develop on their surface during the latter stage.

Up to late mantle descension the filaments are solid. Mesoblasts form the core in each. In young adults the filaments become hollow (fig. 21a). On the ventral floor of each lumen a double supporting rod is secreted. After hatching occurs, each filament elongates dorsoventrally (*cf.* figs. 21a, 21b), a solid growth of cells occurs downward from the level of the supporting bar, and the lumen in the dorsal part of the filament is greatly elongated. The bifurcate rod is thus in the approximate middle of each filament (fig. 21b, *sr*). At the base of the filament the two halves of the bar fuse to form a single bar embedded in the mantle wall.

Mantle.—The pocket formed when the combined anlagen of the ctenidium and endostyle lip over the ectoderm in front of the ctenidial ridge is the mantle cavity (figs. 5a, 18, *mc*). Along with the anlagen of the ctenidium and endostyle, the mantle cavity is carried forward and from right to left (figs. 5–7, *mc*). During late mantle ascension, the mantle cavity lies vertically on the left side (fig. 6, *mc*). As the yolk is utilized, the yolk cells recede, the mantle widens to the right and eventually covers the entire cephalic region as the shell descends. Into the mantle fold are carried the excretory organ, the intestine, and the pericardium (figs. 10, 11). The sheet of cells on which are situated the endostyle and ctenidium doubles back on itself to form the ventral face of the mantle fold or the roof of the mantle cavity (*cf.* figs. 6, 7a, *en*). The dorsal face of the mantle fold lies beneath the anterior half of the shell.

As the shell descends, the shell gland retreats beneath the periphery of the shell and becomes the periphery of the mantle fold. At the sides of the animal, the periphery of the mantle fold is continued beneath the shell edge around the posterior half of the individual as the accessory mantle fold (fig. 20, *amf*). The cavity between the accessory mantle fold and the foot is the accessory mantle cavity (fig. 20, *amc*). The periphery of the shelf lamina is the accessory mantle fold in adults.

DEVELOPMENT OF THE BLOOD SYSTEM

Larval heart and circulation.—Formation of the larval heart begins at the time of movement of the supraoesophageal ganglion from the right to the left (fig. 5, *lh*). The epithelial cells which lie posterior to the head vesicle, to the right of and above the supraoesophageal connective, and anterior to the anlage of the visceral ganglion, become attenuated to form a pulsating membrane, the larval heart, which gradually enlarges, reaching its maximum size during mantle ascension (fig. 6, *lh*). During torsion, the larval heart is carried toward the left side (*cf.* figs. 5a, 6, *lh*), and whereas the earliest position

of the larval heart is right lateral and slightly dorsal, its ultimate position is definitely dorsal (cf. figs. 5a, 7b, 1h).

Circulation of the embryonic fluid is maintained by the larval heart. The cavity of the vesicle connect directly with the cavity of the larva which lies between the external epithelium and the gut. There are no blood vessels. During the stage of mantle descension, the adult heart is ready to take over the function of circulating the blood, and the larval heart is crushed by the pressure of the downward-moving mantle on the dorsal surface of the neck. The membrane becomes a portion of the epithelium of the mantle cavity.

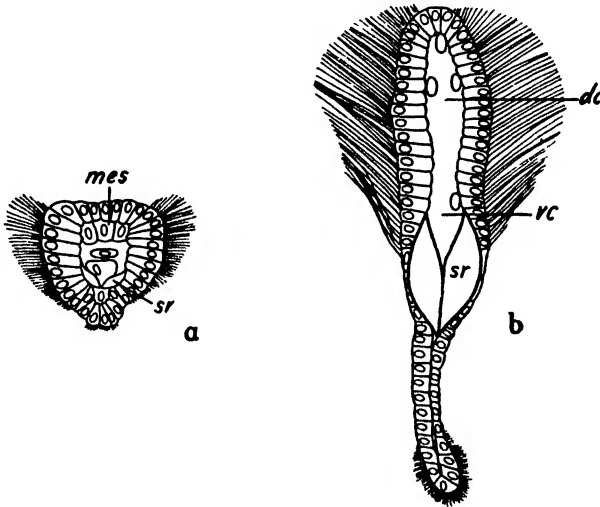


Fig. 21. Transverse sections through ctenidial filaments of: (a) a young adult, (b) a mature male.

Pericardium, adult heart, blood vessels, and sinuses.—Between the stages shown as figures 5 and 6, a sheet of twenty to thirty cells is seen within the hemocoel below the developing ctenidium and endostyle (fig. 18, *pc*). Whether the cells composing this sheet were budded from the excretory cell mass or are an independent group of the original mesoblast cells in that area is not certain. Roughly, the sheet is two cells in thickness. The dorsal set of cells composing the sheet separates from the ventral set during the transition from mantle ascension to mantle descension (fig. 19, *pc*). The pericardial cavity is thus formed, surrounded by a delicate membrane, the pericardium, one cell thick. It lies to the left of the excretory organ and partly beneath the mantle cavity. As development proceeds, the pericardium is successively ventral, posterior, and dorsal to the mantle cavity, the dorsal being its ultimate position.

Formation of the heart takes place rapidly, and some points are in doubt. A solid bud of cells forms within the pericardial cavity (fig. 19, *ht*), develops a lumen, and soon is tubular in character, attached at both ends of the tube to the pericardium. Diagrams of the supposed development of the heart are given in figure 22. At hatching, the tubular heart has constricted in the middle to form an anterior atrium and a posterior ventricle (figs. 22d, 11, *at*, *v*). A

connection between the atrium and the cavity of the mantle fold lying along the bases of the ctenidial filaments has appeared. The exit of the ventricle to the excretory organ and viscera has not developed. The blood system is very incompletely developed at hatching. The heart has not completed its development, and many of the sinuses and vessels have not formed. During post-embryonic development, the ventricle and atrium both become broader than the entire heart is long (fig. 22e, *at*, *v*). The ventricle becomes diamond shaped. The atrium grows laterally and on the left side posteriorly until it reaches

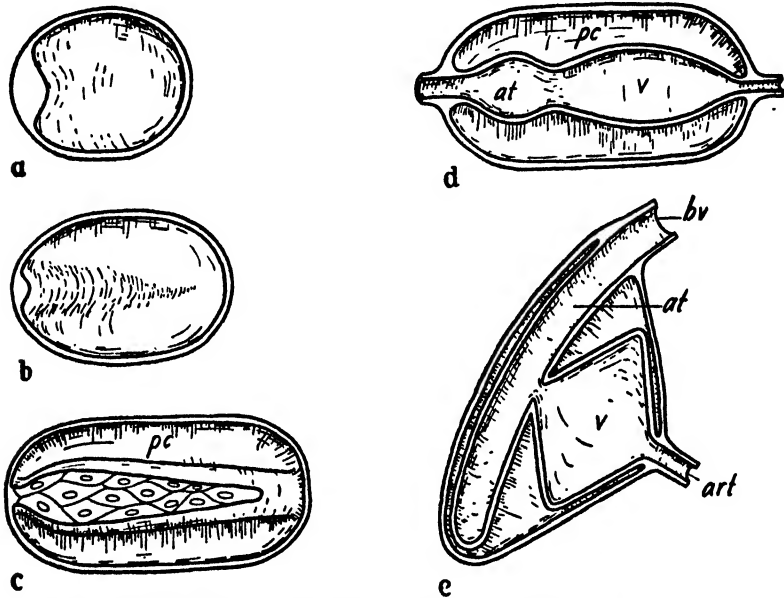


Fig. 22. Diagrams of hypothetical dorsal hemisections of the heart and pericardium at various stages of development.

art, arterial trunk; *at*, atrium; *bv*, branchial vein; *dc*, dorsal canal; *mes*, mesenchyme cell; *pc*, pericardial cavity; *sr*, supporting rod; *v*, ventricle; *vc*, ventral canal.

posterior to the ventricle on the left. Toward the apex of the visceral mass in the adult, the atrium merges into the branchial vein. The cavities of the atrium and ventricle become traversed and their walls strengthened, particularly the ventricular wall, by the increase of muscular fibers, which first can be seen in the young adult stage as delicate myocytes.

Development of the blood vessels and sinuses is best explained by stating that their limits are set by the development of connective and muscular tissues. The vascular ramifications are so diffuse as to make development quite difficult to determine and even more difficult to explain. In *Crepidula* the vessels are limited by delicate membranes which look more like the boundaries of adjacent connective tissue cells than vascular walls.

It is certain, however, that the spaces which constitute these blood vessels and sinuses are present from the time of formation of the blastocoele, which evolves into the adult hemocoele. It is the growth of connective tissue which limits the blastocoele to more or less definite blood spaces.

DEVELOPMENT OF THE MUSCULAR SYSTEM

Larval muscles.—Lillie (1895, p. 38) first used the term myoblast to designate the unicellular muscles of the molluscan larva. In *Crepidula* they are mesoblastic cells, probably derived from ectodermal cells of the second quartette in quadrants A, B, and C. Some may also arise from the dispersal of the mesoderm bands. In the pedal hemocoel they stretch from wall to wall in the void of the vesicle (fig. 15, *my*); their function in this region is probably to keep the delicate head vesicle from bursting. Ultimately they are probably developed into adult muscle cells, particularly the dermal musculature.

Adult muscles.—The various adult muscle groups may be designated as the pedal, the buccal, and the dermal groups. Their development is as follows.

Pedal muscles.—In addition to the attenuated myoblasts in the pedal hemocoel, there is a confused mass of nonattenuated myoblasts, which, in larvae undergoing torsion, resemble the ectodermal cells composing ganglia (fig. 15). These mesoblasts give rise to the adult pedal-muscle cells.

Buccal muscles.—In elongating larvae the early radular sac acquires two bundles of cells on its lateroventral surfaces. These cells are probably mesoblastic in origin. They begin metamorphosis into the pharyngeal and radular muscles during late mantle descension and are fully developed by the time hatching occurs.

Dermal muscles.—Mesoblasts find their way to all parts of the hemocoel. Many of them adhere closely to the external epithelium, to which they become attached. In late mantle descension these mesoblasts, some of which, probably, have already functioned in a contractile capacity as myoblasts, develop into long cells attached to the external epithelium. Such muscle cells are the dermal muscles, which bring about the contraction of the head, tentacles, and mantle.

DEVELOPMENT OF THE SUPPORTIVE SYSTEM

Shell.—Invagination of the shell gland, formed of cells derived from 2d, occurs during the formation of the stomodaeum (fig. 1, *shg*). The gland lies posteriorly, to the left of the mid-line, slightly above the midfrontal plane. Evagination of the gland does not occur. Secretion of the shell begins after invagination is fully accomplished (fig. 2, *sh*), and the gland and shell increase concentrically (figs. 3 et seq., *sh, shg*). At first blisterlike, the shell soon caps the left posterior region of the larva and later becomes dome shaped (fig. 4, *sh*). Some authorities deny a relation between torsion and coiling; this may or may not exist. It is significant, however, that in *C. adunca* the twist of the shell begins during torsion (fig. 5b). The shell peak is carried to the right and twisted in a counterclockwise direction, the posterior being taken as the point of observation. In the stage of the appearance of the larval heart the shell is no longer symmetrical in contour. It lops noticeably toward the right with a tendency to hook upward. This wry condition of the shell is illustrated in figure 5b, where the apex (*sha*) may be seen on the right side of the larva.

Growth of the shell gland into an ever-widening circle is also accompanied by its movement forward, more rapidly dorsally than ventrally. By this concurrent movement the left side of the larva is hard pressed and doubles into a fold (fig. 4a, *sfl*), the shelf lamina. Torsion carries the lamina from its left lateral site to the ventrum of the larva (cf. figs. 4a, 6, *sfl*). It reaches its final position about the time of mantle descension, grows rapidly in a posterior direction during mantle descension, and forms a thick, broad, flat lamina between the shell and foot. It is separated from the foot by the sublamine space (fig. 15, *sbs*), and from the visceral hump by the supralamine space (fig. 15, *sps*). In the time elapsing between late mantle descension and the young adult stage the sublamine space becomes successively shallower as its forward anterior blind end moves posteriorly. Thus the shelf lamina is incorporated into the foot as the dorsal half of the metapodium, and the sublamine space becomes the accessory mantle cavity (cf. fig. 15, *sbs*, and Moritz, 1938, fig. 1, *amc*). The periphery of the lamina is continuous laterally with the shell gland, and it is the periphery which forms the epipodium around the posterior half of the individual. The supralamine space is not obliterated. The shell shelf occupies this space in the adult. Over the entire visceral dome the shell is thick; on the surface of the visceral dome, however, which forms the dorsal wall of the supralamine space, the shell is present as a thin sheet of secreted material. This thin sheet becomes the core of the shelf in the adult. The remaining portion of the shell covering the visceral dome becomes the periostracum. This accounts for all the shell except the rim extending from shelf to substrate, or that part of the shell which circles the metapodium. This rim is secreted by the metapodium.

Young adults have a shell which distinctly shows the results of torsion. A definite suture occurs at the junction of the shelf with the rest of the shell. The rim of the shell between shelf and substrate is secondary, and is a ceno-genetic, not a palingenetic character. The larval shell is not cast off after hatching, but to it an addition is made to build up the adult shell. In young adults the shell has but two layers, periostracum and an inner layer, the future nacreous layer (fig. 20, *pm*, *nc*). Soon after hatching, the third or middle layer, the prismatic layer, is added by secretion at the edge of the mantle. Thus the tip of the adult shell has no prismatic layer. The rest of the shell, except the shelf, is composed of the usual three layers, although the periostracum is frequently lacking because of wear. It is suspected that the shelf is constituted by the nacreous layer alone, but this is not definitely known.

From the development of the shell it is evident that it is the original left side of the larva that secretes the shell shelf. In all probability it is the left side of the larva of *Natica* that secretes the columella. Hence, it seems that the shelf of *Crepidula* is the homologue of the columella of *Natica* and of the coiled shells of other gasteropods.

Connective tissue.—The mesoblasts do not give rise to this important molluscan tissue until the young adult stage is reached; even then it is meager. After the spat hatch, these cells begin a rapid metamorphosis into connective tissue cells which soon occupy much of the perivisceral cavity, confining the blood to the irregular hemocoele.

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Hatching occurs before formation of the reproductive system is complete. The penis is the only unit of the system present at that time (fig. 8, p).

The penis and its ciliated groove.—In late mantle descension a small outgrowth, the anlage of the penis, appears on the head posterior to the right tentacle. It soon develops cilia in a shallow groove on its under surface. At the junction of the penis with the body this groove passes to the dorsal and right side of the neck, where it runs posteriorly. At hatching, or shortly after, the penis has attained considerable size (fig. 8, p). The ciliated groove, U-shaped in cross section, runs along its ventral surface, curves to the dorsal surface at the base of the penis, and runs onto the right neck lappet lateral to the broad, shallow food groove. At the junction of the neck with the body, the groove runs laterally to the edge of the mantle at the anterior margin of the right shell muscle. There its two lips, the sides of the U, meet two other lips projecting from the mantle wall. The four prongs of the two U's meet, and a tube is formed. This is the vas deferens or gonoduct. The uterus is later formed from part of the vas deferens and the unfused anterior portion of the double U channel.

Gonad.—Young adults do not have the primordium of the gonad in evidence. At least, no cells have been identified as being the primordium, according to Gould's description (1917, pp. 11–12). However, soon after hatching, a group of gonadial cells can be seen below the anterior tip of the digestive gland. The origin of these cells is unknown, although it is possible that they arise from the pericardial wall, as in *Viviparus* (Drummond, 1902, p. 99). These cells, migrating ventrally between the right wall of the mantle and the anterior lobe of the digestive gland, reach their ultimate position above the oesophagus at the ventral and anterior tip of the digestive gland. A process of the gonad is sent to the right. It enlarges somewhat at the end and begins growth forward. In a male 2 mm. in length the main gonadial body lies on the right at some distance from its supposed origin, the pericardial wall. A long process extends across the mid-line of the body dorsal to the oesophagus and ventral to the digestive gland to end blindly on the left side. An outgrowth, the gonoduct, extends forward on the right side from the main body of the gonad toward the posterior end of the ciliated groove already described. By the time the male has reached a length of 3 mm., the gonoduct has opened to the exterior. Giese describes a gonopericardial pore for *Calyptraea* (1915, pp. 180–181). Examination of slides and gross dissections have revealed no such pore in *Crepidula adunca*.

SUMMARY

1. *Crepidula adunca* is a protandrous gastropod which breeds the year round along the northern California coast.

2. The young are retained in capsules through the larval stages and when liberated resemble diminutive adults. Development is direct and gradual. A free-living trochophore and veliger are lacking.

3. At hatching, all the definitive organs are present except the gonad.

4. The development of the digestive tract of *Crepidula adunca* is, in general, similar to that in other mollusks. Invagination of the proctodaeum is barely perceptible.

5. The ganglia, commissures, and connectives are formed by a sinking inward from the external epithelium. The pleural ganglia exhibit slight modifications of this process. No outgrowth of fibers from ganglia is apparent in the initial formation of the commissures or connectives, although it is not denied that processes may later be sent into the commissures and connectives from the ganglionic cell bodies.

6. Larval excretory cells, derived from second or third quartette cells, appear about the time the shell gland develops and are cast off from the surface epithelium just before hatching occurs.

7. There is evidence indicating that the adult excretory organ probably arises from derivatives of 2d.

8. The pericardium and heart arise from the same cellular mass as does the excretory organ. Growth of mesenchyme reduces the blastocoele to irregular spaces, which form the hemocoele and blood vessels in the adult.

9. The shell bears a shelf, a structure not commonly found among gastropods. The shelf is secreted by a fold of external epithelium, originally developed on the left side of the larva but subsequently forming the dorsal surface of the metapodium. It is suggested that the shelf is the homologue of the columella of coiled shells.

10. The origin of the gonad is uncertain. It probably is derived from the pericardium.

11. Torsion is demonstrable and results from gradual dissimilar cell growth of the right and left sides. It has been maintained that larval muscles are instrumental in causing torsion in other gastropods, but there is no evidence to indicate such instrumentality in *Crepidula adunca*.

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THE DEVELOPMENT OF THE HEART IN THE RAT

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THE DEVELOPMENT OF THE HEART IN THE RAT

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INTRODUCTION

IT SEEMS REMARKABLE that in all the literature on the development of the heart in mammals there is not a single complete account of this organ from its beginnings to its final division into chambers after birth, investigations thus far having been variously confined to the early stages, or to intermediate stages, or to the later periods when division begins. For only five higher mammals (the rabbit, the guinea pig, the ferret, the cat, and man) and for a few marsupials does the account approach completeness. It has, therefore, seemed worth while to offer this more comprehensive history* of the heart in the rat.

To obtain the substance of this report quickly, easily, and clearly, the plates should first be studied with or without the descriptions accompanying them. The plates alone will give most of what some may wish to get from this paper; with the descriptions one has a nearly complete account of the rat; and for a better understanding of the rat, and for certain comparisons and problems, the rest of the text is necessary. The reader is accordingly referred to the plates and their descriptions (p. 263) before reading the following pages.

The following is a list of authors who have made contributions to the embryology of the heart, arranged according to the animals studied.

Calf: Shaner (1929).

Cat: Schulte (1914, 1916); Watson (1924).

Dog: Bonnet (1901).

Ferret: Wang (1917, 1918).

Guinea pig: Strahl and Carius (1889); Yoshinaga (1921); Elliott (1931).

Man: His (1885); Born (1888); Rose (1889); Retzer (1908); Tandler (1912); Mall (1912); Waterston (1913); Congdon (1922); Davis (1927); Patten *et al.* (1929); Patten (1931); Odgers (1935).

Marsupials (five species): Parker (1915).

Pig: Retzer (1908); Morrill (1916); Streeter (1927); Kellogg (1928); Shaner (1928); Bennett (1936); Goldsmith and Butler (1937).

Rabbit: Hensen (1875, 1876); Born (1888, 1889); Strahl and Carius (1889); Assheton (1895); Rouviere (1904); Bremer (1912); Murray (1919); Girgis (1930, 1933).

Rat: Robinson (1892); Ravn (1895); Adelman (1925); Butcher (1929); Goss (1935, 1938).

Sheep: Bonnet (1889).

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DESCRIPTIONS, COMPARISONS, AND DISCUSSION

EARLIEST STAGE TO 5 SOMITES

Origin of cardiogenic mesoderm, pericardial cavity, and cardiac fundament.—Since Hensen's work (1875) on the rabbit and the guinea pig, the mesoderm from which the heart comes has been recognized as arising, before the advent of the head fold, as separate right and left fundaments which are peripheral, thickened, ill-defined parts of the layer of embryonic mesoderm. This was recognized by Robinson (1892) and figured by him for the rat, although very inadequately. The beginnings and early growth of the mesoderm in the rat, together with the origin of the exocoelome and amnion, have been more fully described by Long and Burlingame (1938). Figures 1*a* and 1*b* show the mesoderm as still separate right and left layers in the embryonic area, *below* the amnion, continuous with each other only through the primitive streak. However, *above* the amnion these two layers are continuous with each other, since they are continuous with the mesoderm lining the exocoelome. This will be clear if one notes that the amnion (fig. 1) is like a membrane or septum separating the amnionic cavity from the exocoelome, and is attached around its periphery to embryonic mesoderm except where that is lacking in the median plane at the anterior end between the parts labeled "cardiogenic mesoderm." As will readily be seen in later figures (2–10), the lateral edge of the cardiogenic mesoderm, as the latter becomes more extensive, is marked by the line of attachment of the amnion. After the right and left parts of the cardiogenic mesoderm unite (fig. 5) across the median line, they have the well-known horseshoe shape.

It is also generally recognized (most recently by Streeter, 1927, for the pig, and by Davis, 1927, for man) that the pericardial cavity originates in this cardiogenic mesoderm by the appearance of small spaces which coalesce to form larger, single, closed right and left cavities which in turn unite in the anterior median line into a single horseshoe-shaped cavity, the future pericardial cavity and possibly a part of the pleural cavities as well. For the rat, Robinson (1892) shows this cavity in sagittal section; Adelman (1925) also figures it in his plate 6 of a 3-somite rat. More recently Butcher (1929) and Goss (1935) have directed attention to the same facts. The rat, then, as figured in this paper is reasonably typical. Goss (1935) says with respect to the pleuropericardial cavity, "The expansion is uneven, however, being greatest at the sides of the anterior intestinal portal and least in the mid line where it becomes extremely small or may be obliterated. . . . The lumen of the central portion again becomes patent but the so-called middle cardiac plate lags behind in its development." Our figures, which show a considerable degree of variation at this stage, agree substantially with Goss's statement, and it may be debatable whether our figure 10*a* shows a late union or an obliteration. In view of the variations, however, it appears to us to be a late union. But our data do not seem to agree with the statement that the "middle plate" lags behind in development, and we find it hard to harmonize Goss's experimental results.

Butcher (1929) finds that the connection between pericardial cavity and exocoelome is established by more than one pore, but we have seen only one, which soon becomes a wide passage.

Myocardium.—Studies on the early stages of marsupials (Parker, 1915), the rabbit (Rouviere, 1904; Bremer, 1912; Murray, 1919; Girgis, 1930), the cat (Schulte, 1916; Watson, 1924), the guinea pig (Yoshinaga, 1921), the ferret (Wang, 1917–1918), and man (Davis, 1927) indicate that the myocardium originates in the lateral cardiogenic mesoderms as right and left fundaments which approach each other and unite from anterior to posterior; that in these fundaments at least the bulbar and ventricular regions are early marked off by grooves (Parker, Schulte, Murray, Girgis, Yoshinaga, Davis); and that of these grooves (bulboventricular) the right diminishes and the left deepens as the cardiac (ventricular) loop is established (Schulte, Murray, Girgis, Yoshinaga [?], Davis). The first appearance of the atria, sinus, and vein fundaments is somewhat differently interpreted by the above-named authors. The first region following the ventricular is considered to be the atrium in marsupials (Parker) in the guinea pig (Yoshinaga, 1921), and in man (Davis, 1927), and as the vitelline veins in the rabbit (Girgis, 1930).

The rat presents some rather striking points of difference. Although the fundaments of the myocardium come from the lateral cardiogenic mesoderms which unite in the median line, it is in the median portion of these fused primordia that the heart begins to be molded (figs. 9–12) as a more or less single or two-lobed structure (figs. 9–18) from the splanchnic layer of the cardiogenic mesoderm (cardiac mesoderm). The sections of the heart shown in figures 9–12 particularly suggest great activity in the cardiac cells, amoeboid rather than mitotic, during this short period of development from $2\frac{1}{2}$ to 5 somites. (For example, in the thirteen sections between figures 12e and 12g there are only about eleven mitoses, including late telophase!) It appears that the area concerned rises up and becomes tucked under around its margins so that the pericardial cavity is carried farther and farther under what becomes the dorsal side of the organ, where the mesocardium soon appears. At the same time, in spite of the irregularities of surface caused by the individual cells, and more or less independently of the single or bilateral appearance of the growing myocardium, regions may be distinguished as labeled (bulbus, etc.). In other words, instead of two separate right and left fundaments with regions marked by folds uniting to constitute a single structure, the single heart (sometimes bilobed) is molded directly from a median source (of double origin) with its regions fairly well marked from the first and becoming more and more distinct (see pls. 34–36).

As will be seen from the illustrations, the several 5-somite specimens show considerable variation. Indeed, it is a little difficult to arrange all in a single series without being somewhat arbitrary. It is just possible that some hearts develop more as single, others more as double or bilobed, structures; that is, hearts may not all follow exactly the same path in their development. It is accordingly suggested that two (or four) such paths may be indicated by figures (a) 1–12, 13, 15, 17, 19 (or 15, 18, 19) and (b) 1–12, 14, 16, 18, 19 (or

14, 17, 18, 19). Whatever the sequence, the hearts take on more and more the forms shown in plates 35 and 36, with well-formed bulbus, ventricular loop, atrium expanding and forming lateral lobes (fig. 26), and sinus regions more nearly resembling the development in man (Davis, 1927, pl. 4) than that in the other forms.

Endocardium.—Paralleling the myocardium, the endocardium is described as having a double origin in marsupials (Parker, 1915), in the cat (Schulte, 1914; Watson, 1924), in the rabbit (Bremer, 1912; Girgis, 1930), in the ferret (Wang, 1917–1918), in the guinea pig (Yoshinaga, 1921), and in man (Davis, 1927), the paired endothelial tubes uniting in the well-known way. No attempt has been made to trace the origins of the angioblast cells or endothelial cells in the rat, as was done in other animals by Parker, Schulte, Watson, and Bremer. However, enough has been uncovered to show that the rat endocardium departs from the method of development characteristic of the other mammals studied. The endothelial cells first noted lie between the splanchnic cardiogenic mesoderm and the entoderm (fig. 6*b*) in the 2-somite stage. Similar scattered cells seem to be present also (although not shown in the photographs) in the presomite stage (fig. 4). These cells become more numerous, and presently (figs. 11*b*, *c*, *d*), by the 2½-somite stage, are assembled in the form of a thin, solid plate of the shape shown in figure 11*b*, widest in the middle where it becomes endocardium. Much as in the origin of the pericardial cavity, crevices appearing early (fig. 11*b*) soon increase in size and give rise to the lumen of the endothelial structures of heart and veins, at first as a closed system, later continuous with the ventral aortae and arches (fig. 14, etc.), and apparently still later with the vitelline veins (fig. 16, and later). As is noted of the myocardium, it seems that the endothelial plate also may be transformed into a single (figs. 14, 16) or double tube (fig. 17), all variable in form and extent of lumen and solid portions. There is considerable space between myo- and endocardia, and the endocardial regions do not correspond exactly to those in the myocardium. What may be considered the bulbar (or ventricular?) region is directly continuous with the ventral aortae. It will be noted that the endocardium and ventral aortae particularly have attached to them variously shaped plates of solid endothelium which lie between the floor of the foregut, after the change in position of the heart, and the roof of the pericardial cavity and above the mesocardium, and which seem to be involved in the further evolution of the other arches (figs. 14, 18–20). Similar endothelium seems to be present in marsupials (Parker, 1915, pl. 1, fig. 4), in the cat (Schulte, 1916, fig. 2; Watson, 1924), in the rabbit (Bremer, 1912; Girgis, 1930), in the guinea pig (Yoshinaga, 1921), and in man (Davis, 1927). As pointed out in the descriptions of plates, these details are corroborated by the injected specimens of slightly later age (pls. 36–38).

That the vitelline veins become continuous with the yolk sac later than at 5 somites is also corroborated by the lack of blood cells in both heart and aortae (pls. 29–33), whereas at about 8 somites, the youngest that could be injected (fig. 25, pl. 36), blood cells are present in both the heart and the aortae (bulbus and aortic arch, fig. 20*g*, pl. 34).

EMBRYOS OF 8-26 SOMITES (10½ TO 11½ DAYS)

We have not determined the exact time of heart contraction or pulsation. According to Goss (1938), it begins at about 9 days, 14 hours; unfortunately, the age in somites is not given. However, with the beginning of circulation at 8 somites other changes become evident. The bulboventricular loop with its variations (pl. 35) becomes well formed and distinct and loses much of its mesocardium (fig. 20). Particularly does the atrium enlarge, so that its myocardium, which in the earlier stages (pls. 30-33) is scarcely more than a pair of slight lobes, now becomes a distinct, flattened region (pl. 34) as though by growth in anterior-posterior length, receiving on its left the opening of the atrioventricular canal, and having on its right a corresponding shoulder (see, also, figs. 26*b*, *c*).

Through the 5-somite embryos there is no clear correspondence between the myocardium and endocardium of the atrium. Indeed, there is little at 8 somites (figs. 20*e*, *f*, 25, 25*a*). The endocardium of the atrium is no more than the confluence of the common trunks formed by the union of vitelline, common cardinals, and umbilical veins, and those common trunks may be considered as the horns of the sinus venosus, or the right and left fundaments of the sinus. What there is of the atrium is connected with the left portion of the ventricular loop by the atrioventricular canal to the left of the median line. There is no right shoulder, as in the myocardium. But as the injections of embryos from 9 to 25 somites show (pls. 36-38), the right shoulder appears as the atrium rapidly expands with right and left chambers. The left gives off the atrioventricular canal, and both are continuous symmetrically with the horns of the sinus.

The wall of the ventricular loop becomes thicker and more trabeculate, and the endothelium conforms more and more to the myocardium by the formation of projections over its surface, as described by Davis (1927) for man. The ventricular portion of the loop by a slight constriction exhibits two parts, which seem to correspond to the later right and left ventricles. With the right the bulbus is directly continuous. The wall of the latter (fig. 33*b*) does not have the trabeculate structure of the ventricle; its endothelium is separated from the myocardium, is distinctly flattened and spiral (fig. 32, etc.), and passes on into the truncus arteriosus, where the endocardial and myocardial constituents are in contact with each other. The truncus ends in the aortic sac (as in man, Congdon, 1922), with which the aortic arches communicate. However, as pointed out above, the ventral ends of the arches seem to be derived in part at least from the endothelial plate, which also gives the impression of outgrowths from the sac, as described by Congdon for man (1922).

Up to the end of this stage of development the heart has no true division into right and left halves, although it shows bilaterality in the sinus and atrium, and, in the ventricular loop, consecutive portions destined to contribute to the definitive ventricles. The bulbus exhibits the spiral form which the later division follows. Meanwhile, two aortic arches have appeared by the 12-somite stage; the third arch appears at from 15 to 18 somites; the fourth

arch, however, develops more slowly, seeming to change little from 18 to about 25 somites.

EMBRYOS OF 28 SOMITES (12 DAYS) OR MORE

The beginning of the 12th day, or the stage of 28 somites, marks the first appearance of the longitudinal division of the heart. Just before this age, at 25 somites (fig. 32*a*), the vessels entering the sinus as a symmetrical group are the vitelline, common cardinals, and the umbilical veins. With the development of the liver and septum transversum the vitelline becomes the hepatic. This, with the establishment and growth of the posterior vena cava, is taken over as part of the latter, of which the umbilical (and ductus venosus) is a tributary. The common cardinals become the right and left anterior venae cavae, representing the two horns of the earliest sinus. The hepatic (and later the posterior vena cava) communicates with the right horn, which accordingly is the larger of the two. The three venae cavae are the vessels to be considered in the account of the changes in the atrium.

Changes in the atrium.—The changes in the atrium consist in its division into right and left chambers, alterations in the openings of the veins, and the formation of valves.

An account of the division of the atrium by septa should be preceded by the reminder that from the first the atrium exhibits a distinct bilobed form (figs. 27–32*a*, 34, 35), this, moreover, apart from any pressure from the bulbus; and that the bilobed tendency increases (figs. 44, 51, 52, etc.) independently of the formation of a septum (or septa), which is located at the narrow median region (see also Odgers, for man, 1935) as though to take advantage of the bilobed form.

A comparison of figures 32*a*, 34, 35, 41, and 44 will show how the two sinus horns, which at first open symmetrically into the atrium through a wide (from right to left) opening almost independently of each other, later come to communicate with the right side of the atrium by a single orifice. The margin (marked +) seems to move to the right, past and under the forming septum primum, cutting off direct access to the atrium of the left horn, so that it must join the right horn only. The left horn, or vena cava, thereby becomes greatly lengthened.

Meanwhile, septum 1, with thickened margin, grows toward the atrioventricular canal, as noted by Born (1889) and Girgis (1930, 1933) for the rabbit, Morrill (1916) for the pig, and Tandler (1912) and Waterston (1918) for man. This septum is a continuous partition from the beginning (figs. 35, 37, 38*a*, *b*, 40). Near the atrial wall, where it first appeared, it becomes thin (fig. 38*b*), and ruptures (figs. 43, 44, 46*a*) to form the primary interatrial foramen with ragged margins, as noted by Waterston (1918). These margins later become regular and smooth, and even thickened (fig. 53).

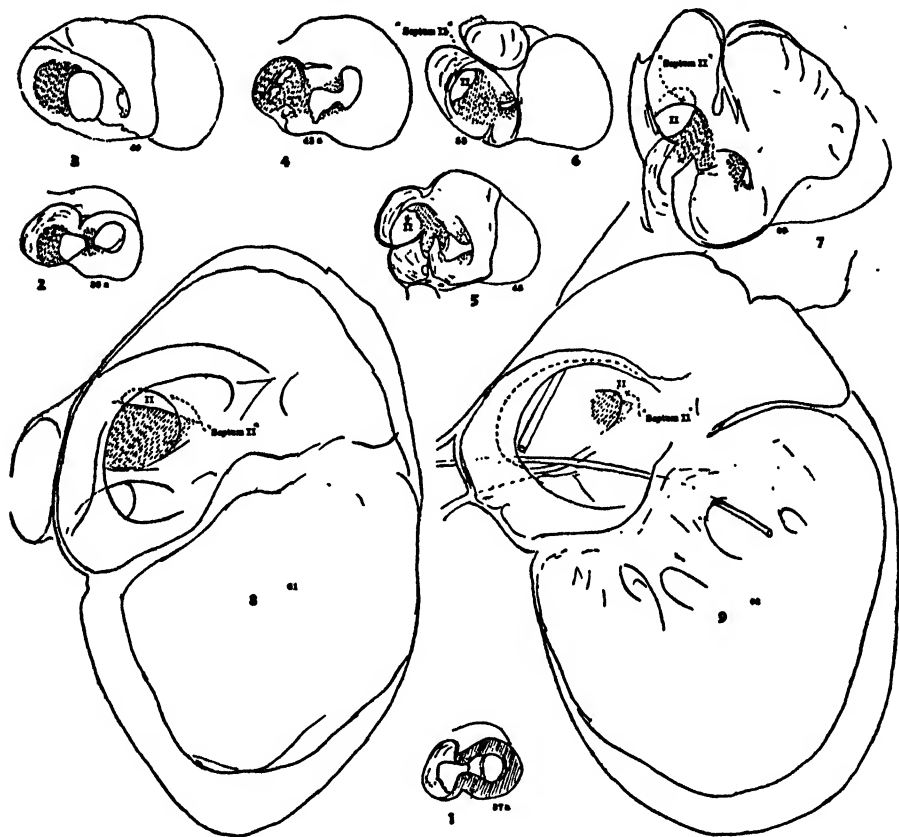
While the septum has been forming, the margins of the sinus opening become elevated or elongated (compare figs. 35, 41, 44, 46, 51*a*, etc.) to form the sinus valve cusps, which to the right unite into, or are continuous as, a single membrane which continues on to the atrium wall as the septum spurium. At

the other end the cusps unite with the base of septum I (figs. 44, 51a, 47). Union or continuity of this kind has been interpreted by some as denoting origin from the primary septum. Figures 41a, 44c, 46, 46a, 47, 48, 51b, 53, 54, and 54a show how the left vena cava opens at the base of the right cusp, in a more and more restricted fashion between the base of the cusp and the margin ("b," especially in 51b) of the post cava. The same margin (labeled "b") is also indicated in figures 57a, 60a, 61d, and 62, which show stages in the process by which the three venae cavae come to open independently of one another into the right auricle, as follows. The post cava and the right anterior vena cava, which unite before entering the right atrium, tend to draw apart, as in figures 61b and 62, yet have a common opening. The separation of the left anterior vena cava begins at 14 days (fig. 51b) with the increase in height of the ridge marked "b" extending from the margin of the posterior vena cava (see also figs. 60 and 60a) to septum I and later (fig. 60a) to the atrium wall in the vicinity of the so-called septum II (figs. 61d, 62). As this septum, or ridge "b," which corresponds exactly to the sinus septum of Tandler (for man, 1912, p. 557), rises still more into the atrium (figs. 60a, 61d, 62) out of the sinus venosus, it seems to move along the right cusp of the sinus valve, and causes the left cava no longer to enter into the sinus, but into the atrium through an opening covered by the lower half or extension of the right cusp. This extension, together with the sinus septum, acts as a valve for the left vena cava, which in man and some other mammals is the coronary sinus. This lower part or extension of the right cusp is the so-called *valvula Thebesii*. The rest of the right cusp is the *valvula Eustachii*, which with the left cusp forms the sinus valve (fig. 62).

At the time that septum I reaches the atrioventricular canal the two atria are separate but for the interatrial foramen or foramen ovale (foramen ovale II). In textbook accounts of the final division of the atrium the advent of another septum (II, or secundum) is generally described on the basis of the early work of His (man, 1885) and of Born (rabbit, 1889). The process, however, is apparently simpler than appears from these accounts, at least in the rat, and probably also in other mammals, and should be reexamined. Septum II is described as arising parallel to septum I; and from two sources in the pig (Morrill, 1916) and in man (Odgers, 1935); also the position of its margin is said to change (Tandler, in man, 1912). It has not been found feasible to attempt to harmonize the various conflicting accounts of the origin and development of the septa and sinus valve cusps. The lack of agreement in previous studies appears to lie chiefly in the fact that the descriptions are based on sections and models reconstructed from sections rather than on the direct observation of whole or dissected hearts. For example, it seems to us that there is confusion in Morrill's and other accounts between the reputed ventral source of the so-called septum II and the sinus septum, and that slight ridges of no significance with respect to septa are magnified to undue importance.

Also, these septa are generally described as coming from the atrium walls and increasing in height as though by their own growth. But one important consideration seems to have been generally overlooked, namely, the growth of

the heart as a whole. In text figures 1-9 are presented outlines, on the same scale, of hearts shown in detail in the plate figures indicated. These outlines are intended only to show the relative sizes of the heart at different stages in growth, the areas of the septum primum (in dash lines) and foramen, their



Figs. 1-9

These text figures are outline drawings, made to the same scale, from figures in the plates, as indicated by the numbers attached. The figures are intended to show the great increase in the size of the heart, the approximate areas of septum I (hatched with dash lines) and its foramen (π), their position and the position of the median atrial walls labeled septum II, and the great expansion of the atria about the foramen as an approximate center. Septum II is considered to be not a true septum, but the median walls of the two atria formed by the deep fold or crease which is the result of the great expansion of the atria. After birth, (9) septum I and foramen II decrease, and the septum becomes thickened and incorporated into the atrial wall.

Figures 1, 2, and 4 are the reverse of figures 37a, 38a, and 43a, to make comparison easier.

position and that of the margin of the interatrial walls. On the one hand it will be seen that the areas of the primary septum and its foramen, even though shown only with approximate accuracy, do not greatly increase up to the end of pregnancy, possibly twice when figures 4 and 8 are compared. On the other hand, the rest of the heart increases enormously, the atria expanding about

the foramen in all directions, the growth on either side of the aorta and pulmonary trunk seeming to increase the height of the so-called secondary septum. This expansion can be seen in face view in figures 44, 51, and 51*a*, and in profile in figures 53 and 60*a*, in which especially (see also fig. 52) the fold in the atrial wall caused by this expansion looks like a septum. Transverse sections through embryos of this age (16 days) and at 17½ days show the fold not to be a septum (II), for it is clearly a deep crease in the wall and structurally is entirely unlike septum I, which is thin and membranous. It seems to us, then, that what is labeled septum II in figures 61*d*, 62, and 62*a* is nothing more than the wall of the atrium; further, that perhaps what is described in other mammals as septum II is not a true septum but only a fold, as shown in the rat. Moreover, this interpretation is in accord with Retzer (for the pig, 1908) and Waterston (for man, 1918).

A comparison of text figures 8 and 9 shows decrease in the area of septum I and of the foramen. Our material does not show the nature of the change in septum I beyond the thickening (compare figs. 61*e* and 62*a*). It looks as if the septum were being incorporated in the wall of the atrium by fusion and histological change, whereby the foramen becomes greatly reduced.

The much greater size of the post cava when compared with the foramen ovale in the late foetus (figs. 61*d*, *e*) is quite in agreement with what Patten, Sommerfield, and Paff (1929) found for the human foetus.

The clusterlike growths in figures 34, 35, and 37*b* (marked "a") and easily seen in other figures at various places on the septa, etc. (41, 43, 44*a*, 57) are of unknown significance and need further study.

Atrioventricular canal, ventricle, and bulbus.—Preparation for the division of the heart starts simultaneously in the atrium, atrioventricular canal, ventricle, and bulbus (figs. 37*a*, *b*, 38*b*, 39*a*), the atrioventricular canal cushions being involved or associated with the other three septa. Although no attempt is made to follow in detail the transformations of the endocardial cushions (see Mall, man, 1912), the general course of events can be traced profitably.

There are the usual four cushions (figs. 39, 39*b*, 42, 50, etc.), the two large middle ones acting chiefly as valves (figs. 46–49). Although these two cushions unite to divide the atrioventricular canal into right and left by the formation of an intermediate mass, this union does not take place until the margin of septum I meets and fuses with them (figs. 46, 47, 48, 53, 56, 60*c*). Meanwhile, the interventricular septum approaches and at about the same time unites with the posterior cushion (figs. 53*a*, etc., 50*a*, 56, 58*b*) a little to the right of its middle point.

Before a view is had of the conclusion of the processes at the atrioventricular division, the bulbus must be inspected. Division of this part begins with the truncus arteriosus, which up to and including figure 38*b* is still single, the bulbar cushions being just barely distinguishable (fig. 38*b*). The details of the division of the truncus are not known (see Congdon, 1922, for man), but it has been accomplished in the stages shown in figures 46*a*, 47, 48, and 55

distal to the two cushions, which are conspicuous spiral swellings that become more and more prominent (figs. 50, 53's, 54, 58's) as the forerunners of the aortopulmonary septum. These cushions seem to correspond to the proximal bulbar swellings of Tandler (man, 1912). By their union (fig. 58) they become the definitive aortopulmonary septum (fig. 59c). However, before this union becomes complete, the cushions establish certain continuities (substantially as described for man by Mall [1912]), as follows.

One of these cushions unites with the interventricular septum (figs. 46a, 47, 48, 50, 53a, 58b, 59c) in the sense of becoming continuous; the other might be considered in the same way as uniting with the right atrioventricular cushion (figs. 50, 50a) or the right ventricular wall near the cushion. At the same time, union between the interventricular septum and the posterior cushion continues in the direction of the bulbus, and of the arrow in figure 58a, so that the anterior cushion becomes involved (figs. 58a, 59-59d). At the stage shown in figure 58 the two trunks derived from the bulbus still open out directly from above the right ventricle, but because of the interventricular foramen, blood from the left ventricle can also escape into both trunks, although it can pass more directly into the systemic. The final closure makes permanent this escape of blood from the left ventricle exclusively to the systemic, and also shuts off the right ventricle from the systemic but leaves it open to the pulmonary. With the advance of the interventricular septum across the anterior cushion there is left only a small opening for the final closing. This opening is indicated by the dotted line (marked X in fig. 58b) in figures 58-58b. It will be seen that the margins of this opening involve the two bulbar cushions, the margin of the interventricular septum, the anterior atrioventricular cushion, and a part of the right wall of the right ventricle at 15 days. A half-day later, when the opening is nearly closed and the bulbar septum is complete, the position of the opening shown in figure 58 is as indicated by the dotted line (X) in figure 59b. The final separation of the ventricles in this manner results in a direct channel between the right ventricle and the pulmonary artery, and a jog between the left ventricle and the aorta, the jog being the interventricular foramen (the "vestibule" of Mall, man, 1912), which is not closed but is incorporated in the aorta.

No attempt has been made to follow the development of the semilunar valves in detail, or to study the vascularization of the heart itself.

SUMMARY

1. The cardiogenic mesoderm arises as separate right and left fundaments which unite in the anterior median plane.

2. In these separate fundaments the pericardial cavity appears as the result of the union of isolated spaces into right and left closed cavities which in turn unite to form a single closed horseshoe-shaped cavity.

3. The myocardium is formed by molding of the central portion of the cardiac mesoderm (the splanchnic part of the cardiogenic mesoderm) largely as the result of cell activity other than mitosis. It is not of separate right and left parts, but is single and median, yet often bilobed.

4. Endothelial cells, which may be recognized before the union of paired pericardial cavities and before the molding of the myocardium, come to form a single thin solid plate from which are derived the endocardium, ventral aortae, perhaps part of the aortic arches, and possibly part of the vitelline veins.

5. The atrium is early indicated by slight paired elevations of the myocardium just posterior to the loop. At this time the atrioventricular canal receives the horns of the sinus, which receive the vitelline, umbilical, and common cardinal veins.

6. With the growth of the atrial myocardium in length, the endocardium exhibits two lobes, the left receiving the atrioventricular canal. The lobes seem to appear independently of pressure from the bulbus.

7. The division of the atrium is accomplished in part by the formation of the septum primum, in which the foramen ovale is formed by irregular rupture.

8. There is no true septum II. It is represented by a deep crevicelike fold of the wall of the atrium caused by great expansion of the atrium on either side of the bulbus.

9. After the sinus opening shifts to the right atrium, the left anterior vena cava or common cardinal acquires a separate opening into the atrium by the rise of a fold which corresponds to the sinus septum.

10. The atrioventricular canal cushions unite, and divide after septum I reaches and unites with them.

11. The final separation of the two ventricles by union of septa, et cetera, is graphically and completely demonstrated.

12. The closure of the foramen ovale is accomplished by fusion of the edges of the septum primum to the wall of the atrium intervening between atria.

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EXPLANATION OF PLATES

THE FIGURES illustrating this paper are stereoscopic photographs, and the user should study them as such in order to understand fully the difficult three-dimensional structures depicted and described. Since the figures are larger than can be placed within the ordinary interpupillary distance, it is necessary to use a stereoscope.¹

All figures bearing the same number but with different letters belong to the same embryo. Each embryo has its own number. The letters attached to the numbers indicate, for the most part, advancing stages of dissection or different views of the same dissection. However, in plates 26 to 33 some photographs appear two or three times under different letters where structures projected as white lines obscure surface details.

All specimens shown in the first nine plates after dissection were carefully oriented and sectioned. Certain structures, such as the mesoderm, cardiogenic mesoderm, pericardial cavity, and endothelium, were then projected three-dimensionally in their proper places on one or more of the photographs. Where figures of some of these sections are included, they are oriented on the plates to correspond as exactly as possible to the photographs, and their locations are indicated by lines on one or more of the other figures. Except on plate 34, photographs of sections are paired to facilitate comparison with the dissections.

The technique of dissecting, photographing, and so on, has already been described (see Long, 1936, and Long and Burlingame, 1938).

¹ A simple form of stereoscope can be obtained from the University of California Press or from the California Laboratory Supply Company.

PLATE 26

Fig. 1. About 9 days. $\times 45$. Egg cylinder dissected from the left almost to the median plane and therefore showing lateral mesoderm. The extraembryonic coelom is in an early stage of expansion. Right and left embryonic mesoderms (below the amnion) are still separate.

Figs. 1a and 1b. $\times 150$. Sections at the levels indicated on figure 1.

Fig. 2. About 9 days. $\times 45$. Dissection similar to that in figure 1. ~

Fig. 2a. $\times 45$. Dorsal view of anterior half of embryonic area. Anterior and mesial boundaries of mesoderm shown in white lines, and minute cavities in anterior part. The mesoderm is continuous with that lining the extraembryonic coelom, as can be seen in figures 2b and 2c. Minute vesicles of unknown significance appear anterior to the cardiogenic mesoderm at the base of the amnion (figs. 2a, b). They may also be seen in figures 3b, c, 6b, 9c.

Figs. 2b and 2c. $\times 150$. Sections through right half of embryo.

Fig. 3. About 9 days. $\times 45$. Dissection from right side.

Fig. 3a. $\times 45$. Dorsal view.

Fig. 3b. $\times 45$. Same as 3a. Shows boundaries of mesoderm, et cetera. The cardiogenic mesoderm is not sharply marked off from the rest of the mesoderm.

Fig. 3c. $\times 150$. Parasagittal section.

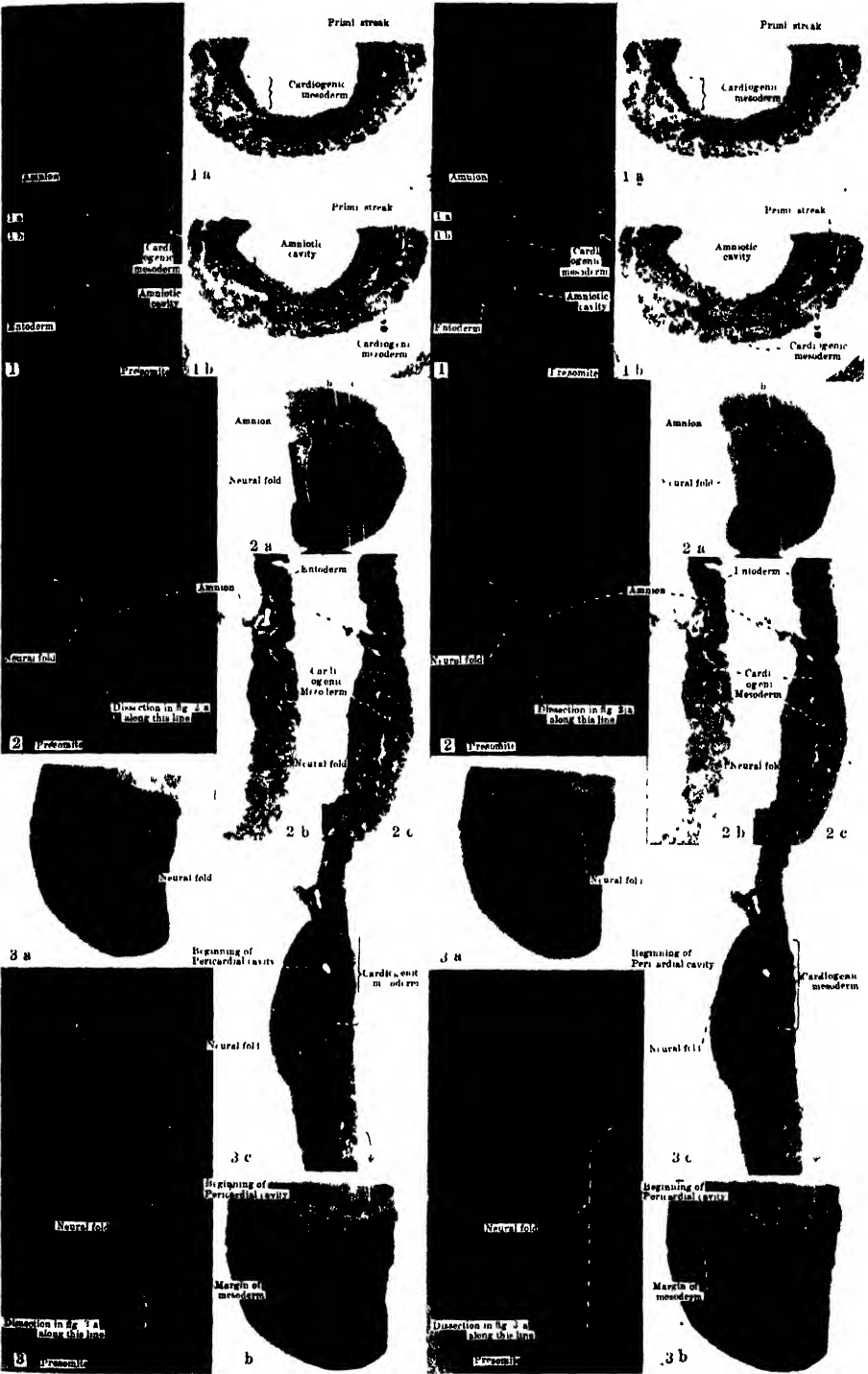


PLATE 27

All dissections are dorsal views like those in figures 2*a* and 3*a*. They show a slight increase in definiteness in the demarcation of cardiogenic mesoderm from the rest of the middle layer; also variations. Dotted lines mark the approximate boundaries of cardiogenic mesoderm, and solid white lines the outlines of the pericardial cavities.

Fig. 4. 9¼ days. × 45. Photographed in creosote. No evidence of somites. Right and left mesoderm plates still separate.

Figs. 4*a* and 4*b*. × 150.

Fig 5. About 9¾ days. × 45. Two somites indicated but not cut off. Photographed in creosote.

Fig. 5*a*. × 45. Photographed in creosote. The right neural fold, and also probably some mesoderm, have been dissected off. The right and left cardiogenic mesoderms are now united across the median line. Right and left pericardial cavities are larger by the confluence of smaller spaces.

Fig. 6. Two somites. About 10 days. × 35. Photographed in creosote.

Fig. 6*a*. Right neural fold removed. Photographed in creosote.

Figs. 6*b* and 6*c*. × 150. Endothelial cells first clearly distinguishable.

Fig. 7. Three somites. × 35. Photographed in alcohol.

Fig. 7*a*. Same as 7, but photographed in creosote.

Fig. 8. Two—three somites. × 45. Photographed in alcohol.

Fig. 8*a*. Same as 8. Photographed in creosote.

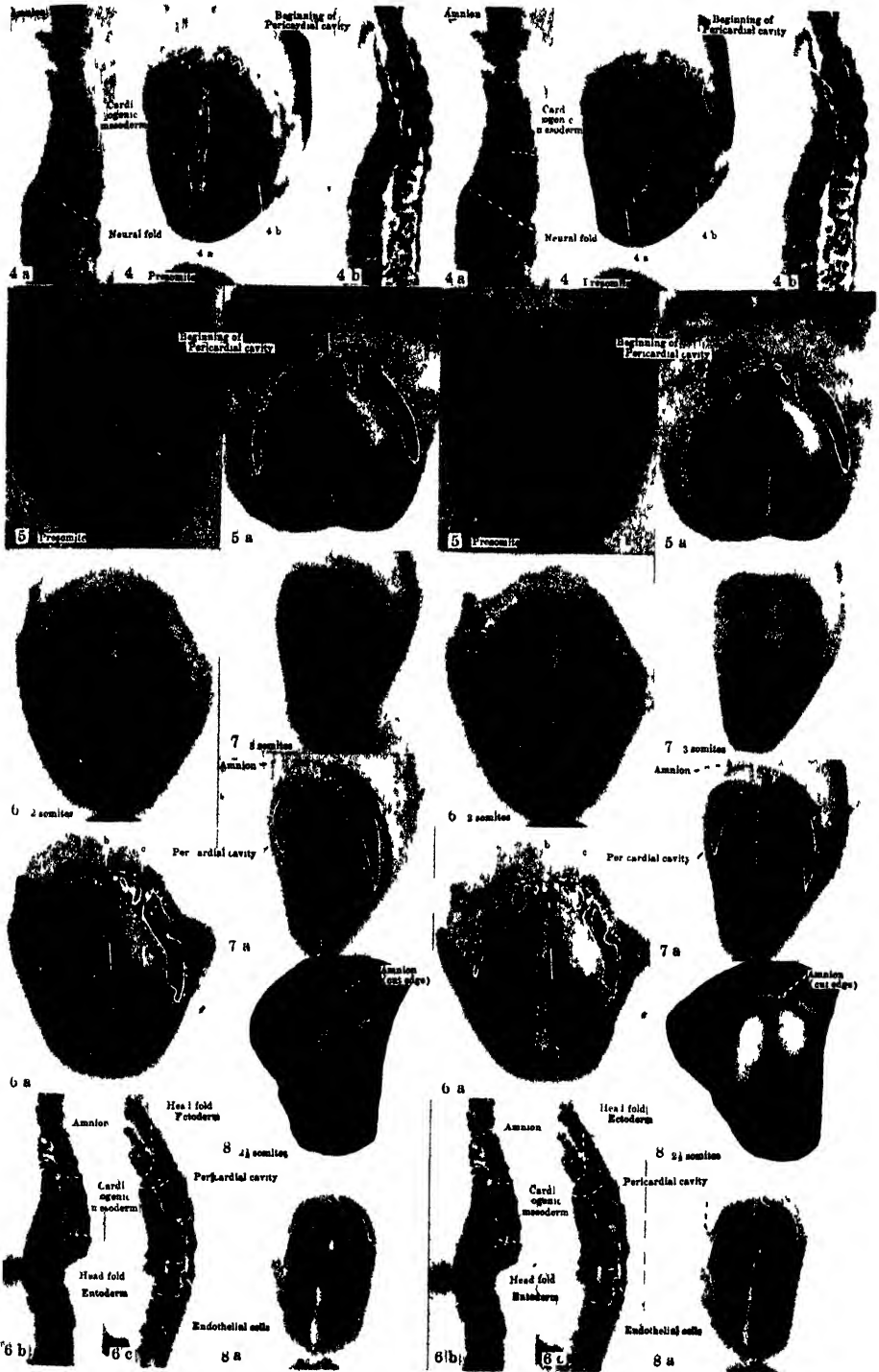


PLATE 28

Fig. 9. $\times 45$. Dorsal view. Photographed in creosote. Anterior part of right neural fold removed to show, if possible, pericardial mesoderm. Only slight indication of latter, as shown by comparison with 9a.

Fig. 9a. Same as 9. Right and left pericardial cavities (shown by solid white lines) have now united across the median line to form a single, closed, horse-shoe-shaped cavity. Dotted lines, cardiogenic mesoderm.

Figs. 9b and 9c. $\times 150$. Left parasagittal and median sections. Splanchnic mesoderm, which is the fundament of the heart, is thicker than the somatic, or primordium of the pericardial mesoderm. Endocardial cells between mesoderm and entoderm.

Fig. 10. $\times 45$. Photographed in creosote. Dorsal portion of neural folds removed.

Fig. 10a. Same as 10. Dotted lines show approximate boundary of cardiogenic mesoderm; solid lines, the pericardial cavities, which in this specimen are still separate, although complete.

Fig. 11. $\times 45$. Photographed in alcohol. Dorsal view of middle part of embryo, which is also an anterior view of head portion. The tips of the neural folds and foregut have been removed by transverse cuts, and the somatic mesoderm and overlying ectoderm have also been cut away, leaving the splanchnic or cardiac mesoderm (of the original cardiogenic fundament) uncovered.

Fig. 11a. The same, photographed in creosote.

Fig. 11b. The same as 11a. Solid white lines show the boundaries of the pericardial cavity dissected wide open anteriorly; dotted lines, the outlines of the endothelium, which is in the form of a thin plate, solid except for two minute cavities. The plate shows several regions, cardiac and venous. The pericardial cavity, which up to this time was closed, now opens by two minute pores into the extraembryonic coelom.

Figs. 11c and 11d. $\times 150$. The somatic mesoderm and ectoderm and the head fold (shown in place in a similar specimen in figs. 9b, c) are lacking. The cardiac mesoderm is thicker and is beginning to take on the form of the heart. The endothelium is platelike.

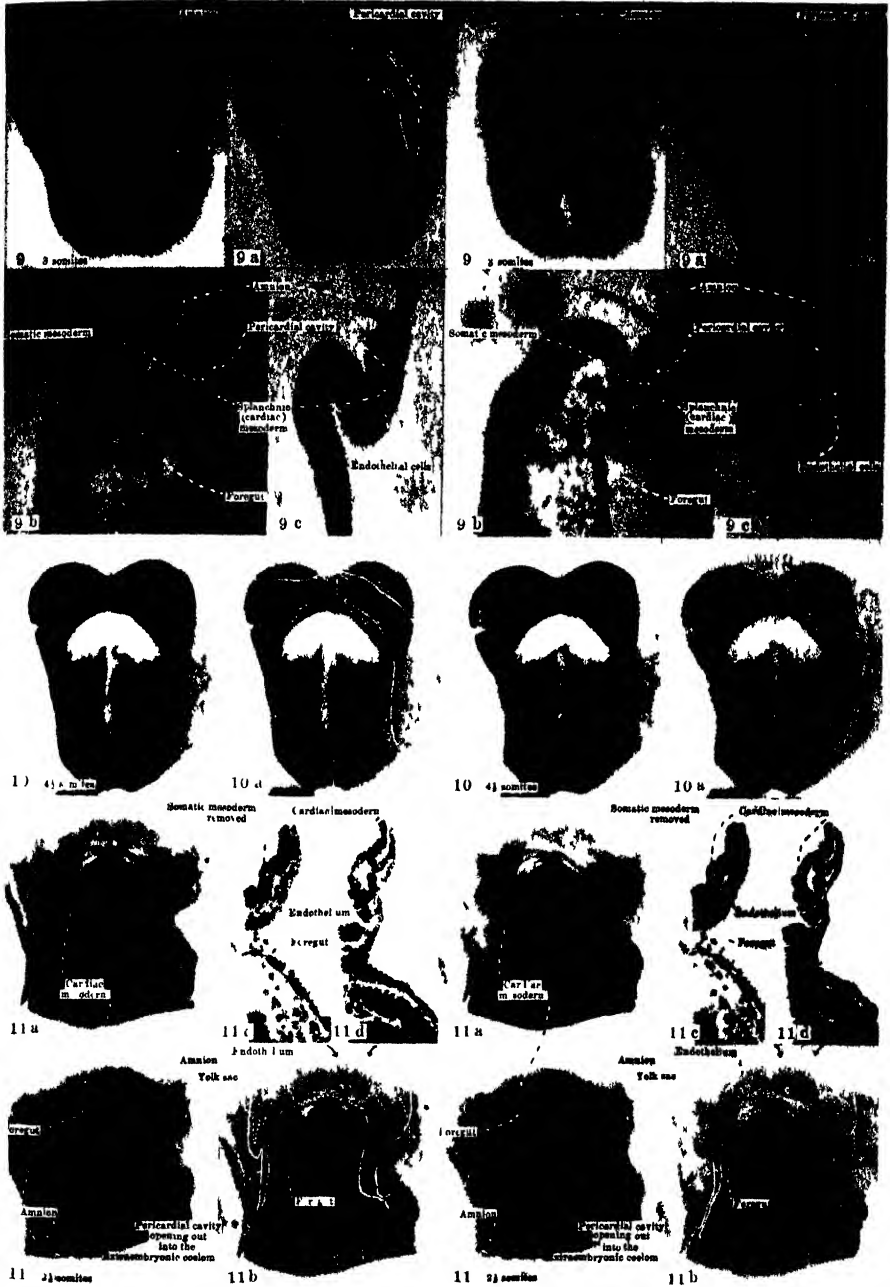


PLATE 29

Fig. 12. $\times 45$. Ventral view of heart region. The anterior parts of the neural folds, the amnion, and the somatopleure covering of the heart have been removed, leaving the pericardial cavity wide open and the growing heart exposed.

Fig. 12a. The same, but seen from a slightly more posterior position, that is, in a view more directly ventral to the anterior intestinal portal.

Fig. 12b. Photographed in creosote. Dorsal view, with all the anterior neural folds and tip of the foregut dissected away.

Fig. 12c. The same as 12b. The solid lines show endothelium with lumen; the dotted portions indicate still solid endothelial plate.

Fig. 12d. Same as 12b, but more directly dorsal. Solid lines are outlines of pericardial cavity, which connects posteriorly with the extraembryonic coelom by wide openings. Comparison with 12c shows that the endothelium is ventral to the pericardial cavity.

Figs. 12c, f, g. $\times 150$. These sections, when compared with the dissections, especially in figures 12 and 12a, show the molding (self-molding?) or transforming of the cardiac mesoderm into the heart, by its growth, modeling, and the tucking under of its peripheral parts, so that the pericardial cavity comes to extend around the heart. Thus the heart tends to rise above the rest of the splanchnic mesoderm. It is just possible that future regions of the heart may be identified, as in figure 12a. No ventral mesocardium.

Fig. 13. $\times 35$. Ventral view. Yolk sac, amnion, and body wall over heart removed.

Fig. 13a. Dorsal view with cephalic neural folds and part of foregut cut away, exposing heart from the dorsal side. The heart shows right and left prominences but not separate fundaments.

Fig. 13b. Same as 13a. White lines are boundaries of pericardial cavity; black lines show the endothelium, which now has a continuous but closed lumen. It is probable that the two prominences containing enlargements of the endocardium represent the beginnings of the right and left ventricles.

Fig. 13c. $\times 150$. Right parasagittal section. The heart is more prominently raised than in figure 12, and the pericardial cavity extends farther around it, thus reducing the extent of the connection between the heart and the splanchnopleure.

At this point attention is directed to the orientation of the growing heart. In figures 9c and 11c the heart is still largely anterior to the foregut and portal, with the free surface of the cardiac mesoderm directed dorsally. With the advancing development of the foregut the whole cardiogenic complex is rotated (as is well known) about a horizontal axis, so that the free surface of the cardiac mesoderm comes to be directed first anteriorly, then ventrally. The beginning of this rotation can be seen in plate 29, and its continuation easily followed in plates 30-33, especially in the sections. The attached side of the heart, and therefore the mesocardium, comes to be dorsal.

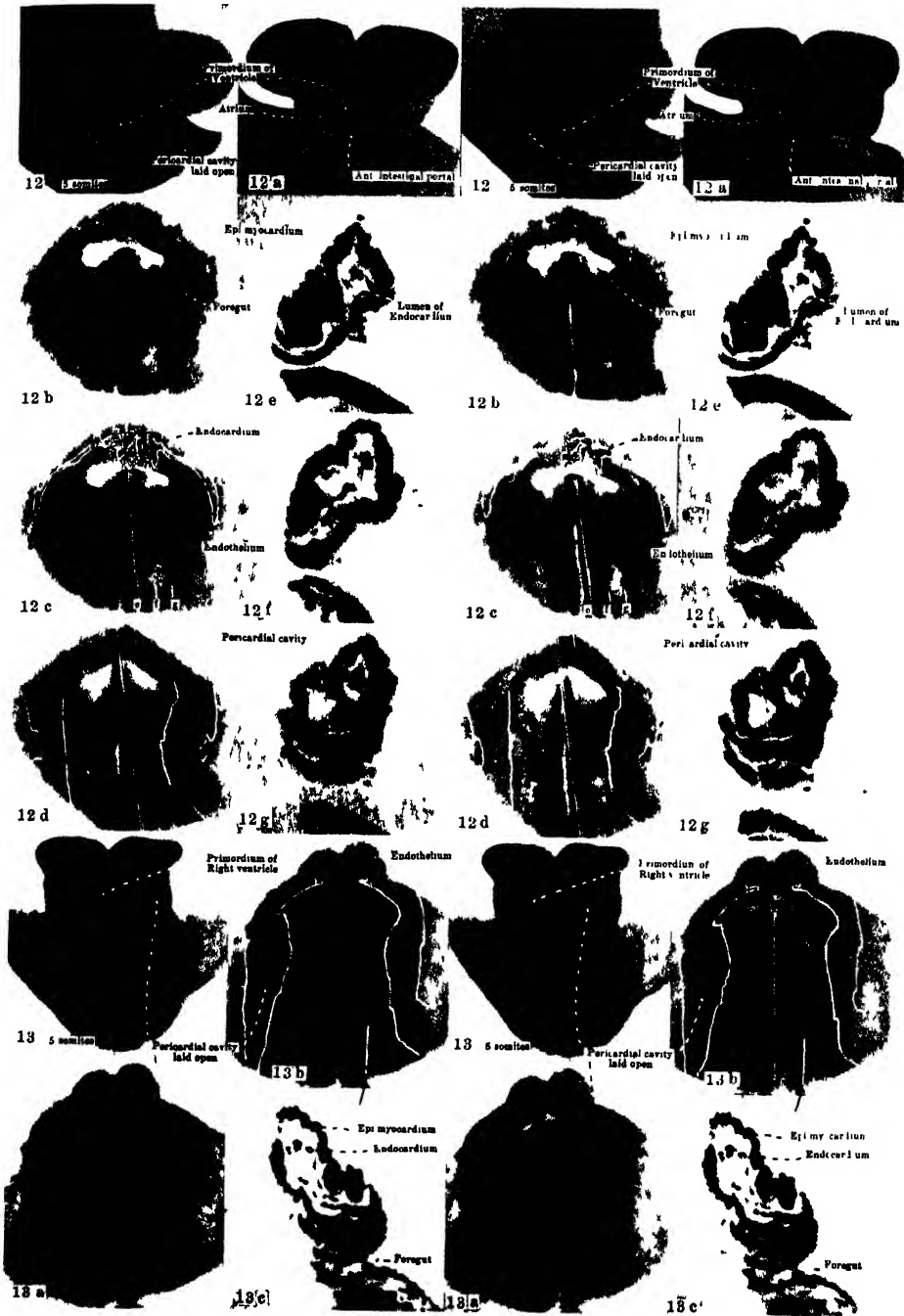


PLATE 30

Fig. 14. $\times 45$. Ventral view, with yolk sac, amnion, and body wall over the heart removed. The white lines indicate the extent of the pericardial cavity both laterally and mesially, and therefore the mesocardium.

Fig. 14*a*. $\times 45$. The heart further exposed. Anterior neural folds cut off. Viewed more anteriorly than in figure 14.

Fig. 14*b*. Same as 14*a*. Pericardial cavity and especially the mesocardium viewed otherwise than in figure 14.

Figs. 14*c*, *d*. $\times 45$. Heart uncovered still more and viewed from more extreme anterior position. *d*. The endothelial plate has become transformed into endocardium, ventral aortae, and the lining of the venous portion of the heart, or has contributed to them. The dotted parts indicate solid masses of endothelial cells. The lumen is continuous only with that of the aortae. The venous end is still closed.

Figs. 14*e*, *f*, *g*. $\times 150$. Epimyocardium still a single layer.

Fig. 15. $\times 45$. Ventral view of exposed heart.

Figs. 15*a*, *b*. The same photograph. In *a* the white lines show the outlines of the pericardial cavity; in *b*, the endothelial system of the heart and attached vessels. The venous portion is closed.

Fig. 15*c*. $\times 150$. The section passes just to the right of the mesocardium.

In this plate and in plates 31, 32, and 33, all of which show embryos of 5 somites, it will be noted that there are considerable differences in the form of the endocardial and myocardial constituents, and also in the state of development. Probably as in the younger specimens, there is no strict correlation between number of somites and state of development of the heart. It would seem that the processes are rapid and perhaps not strictly alike in all embryos, for it is a little difficult to make a single series of all the specimens. As stated in the text on page 251, it may be that the changes in different embryos may follow slightly different paths.

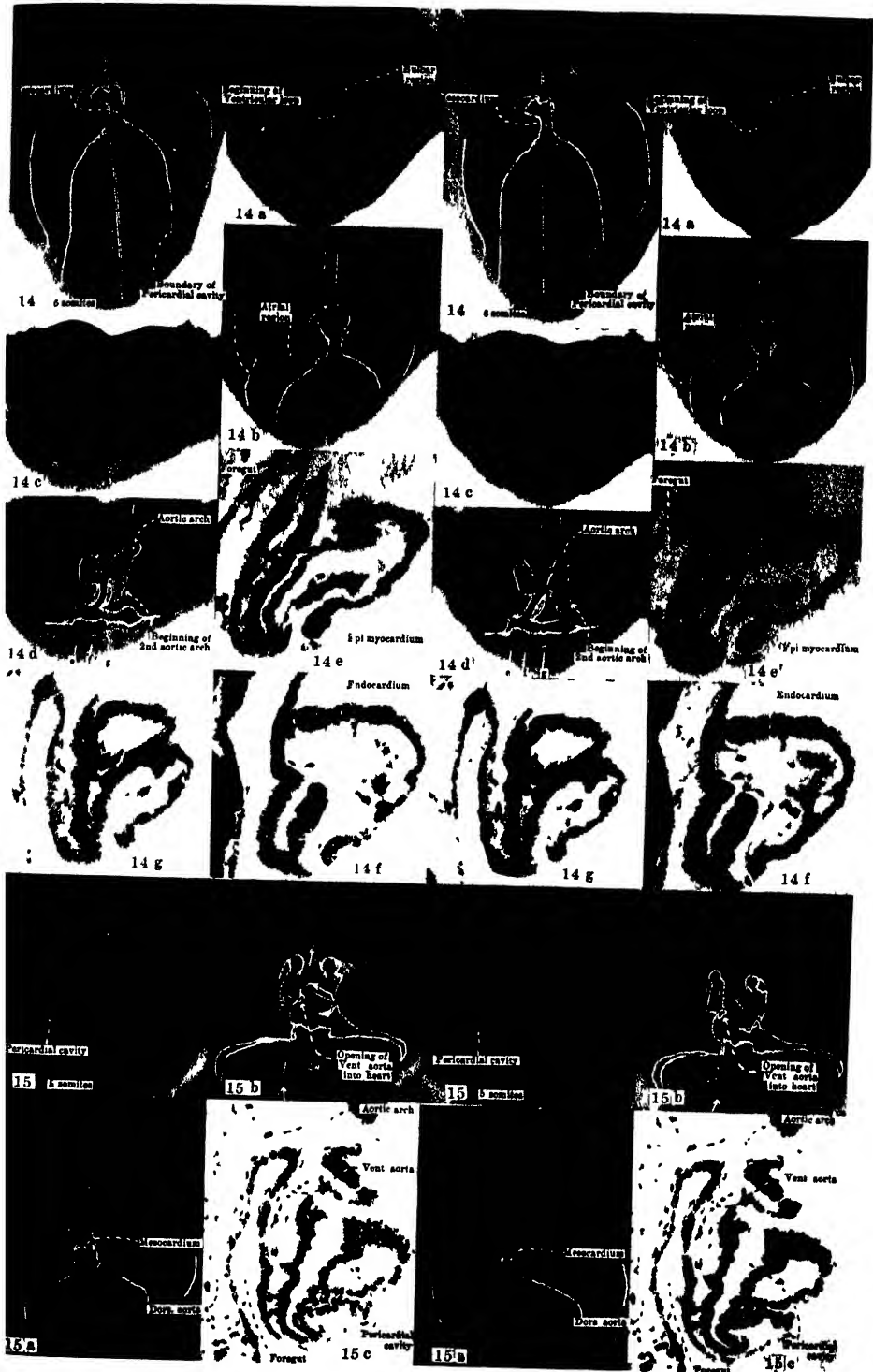


PLATE 31

Fig. 16. $\times 45$. Ventral view of exposed heart. Mesial boundary only of pericardial cavity is outlined in white to show mesocardium.

Fig. 16a. $\times 45$. Same as in 16, but more anterior view. Possible or probable regions of future heart indicated.

Fig. 16b. Same as 16a. There is a considerable amount of solid endothelium intervening between the single ventricular region and the ventral aortae. The lumen of the veins is not complete near the heart, but may be continuous with those forming in the yolk sac.

Figs. 16c, d. $\times 150$. Both show solid endothelial plate. In *d* the plate and ventral aorta are continuous with each other.

Fig. 17. $\times 45$. Ventral view. Heart partly exposed.

Fig. 17a. $\times 45$. Heart more uncovered and in more anterior aspect. As in figure 13, it consists of rather distinct right and left enlargements.

Fig. 17b. Same as 17a. The endocardium also shows right and left chambers. Solid endothelial plates flank the heart and ventral aortae, and the venous regions are solid in part and may possibly be open posteriorly (or laterally).

Figs. 17c, d. $\times 150$.

PLATE 32

Dissections $\times 45$, sections $\times 150$.

Fig. 18. Ventral side of heart exposed.

Figs. 18*a*, *b*, *c*, *d*. More advanced dissection in different views. In *b* the dissection is carried slightly farther on the left side.

Fig. 18*e*. Same as 18*d*. Very little of the endothelial plate left. Second aortic arch appearing on the right side. Endothelial lumen continuous. A ventricular loop seems possibly more evident in the endocardium than in the myocardium. Considerable space on the left side between endo- and myocardia.

Figs. 18*f*, *g*, *h*. One median and two lateral sections.

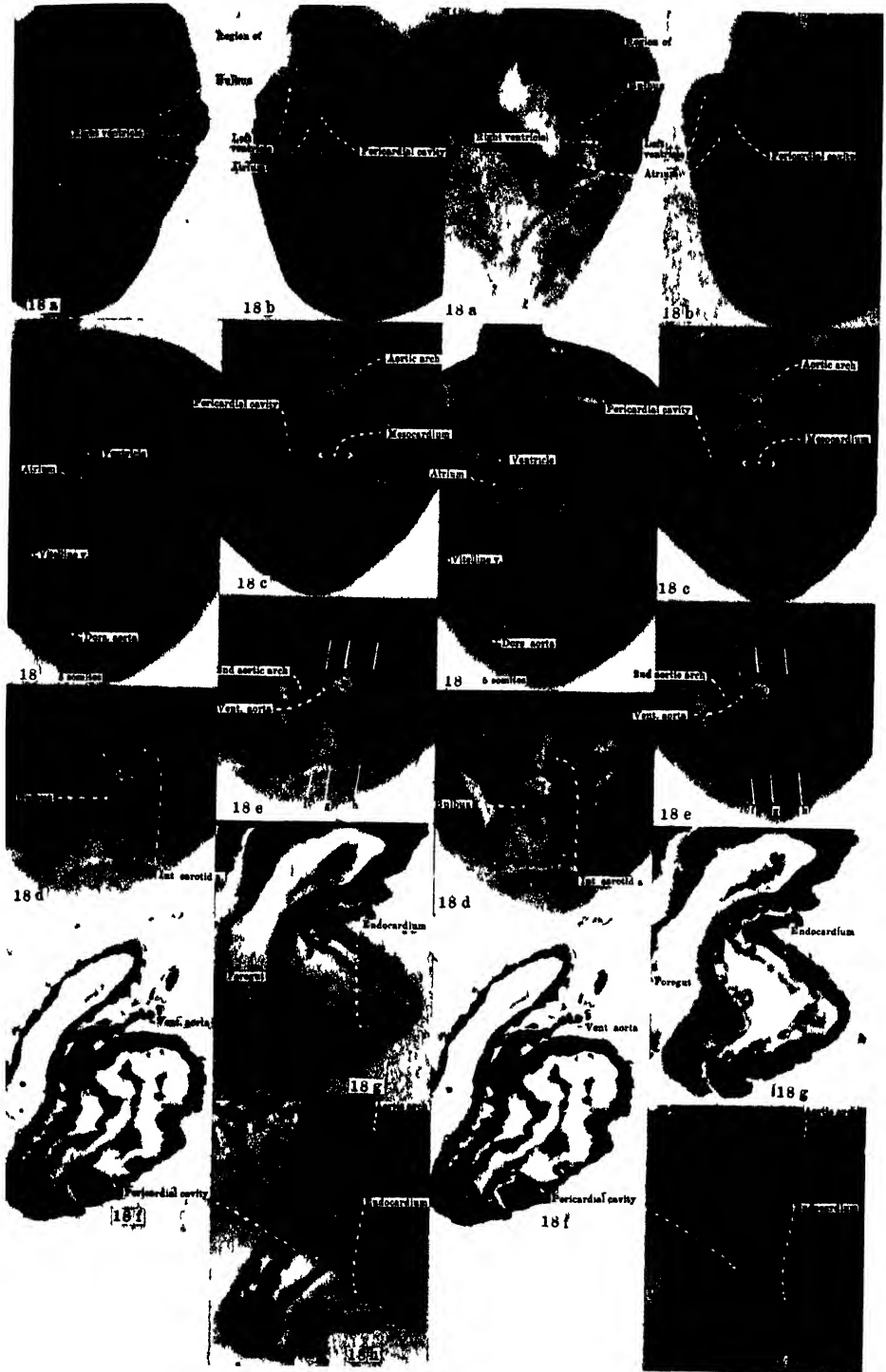


PLATE 33

Dissections $\times 45$, sections $\times 150$.

Fig. 19. Ventral surface of heart uncovered.

Figs. 19a-g. Two further stages of dissection shown in different views. 19*b* is like 19*a*. It shows the endocardium in ventral aspect, the veins being closed distally. 19*g*, a slightly less advanced dissection, portrays the thin mesocardium. 19*c* and *d* are left and right views, respectively. The bulboventricular loop is more conspicuous than in the other embryos of the same age. 19*e* and *f* are duplicates, *f* showing in anterior view the endothelial portions not easily portrayed in ventral view in *b*.

Figs. 19*h* and *i* pass to left and right of the mesocardium.

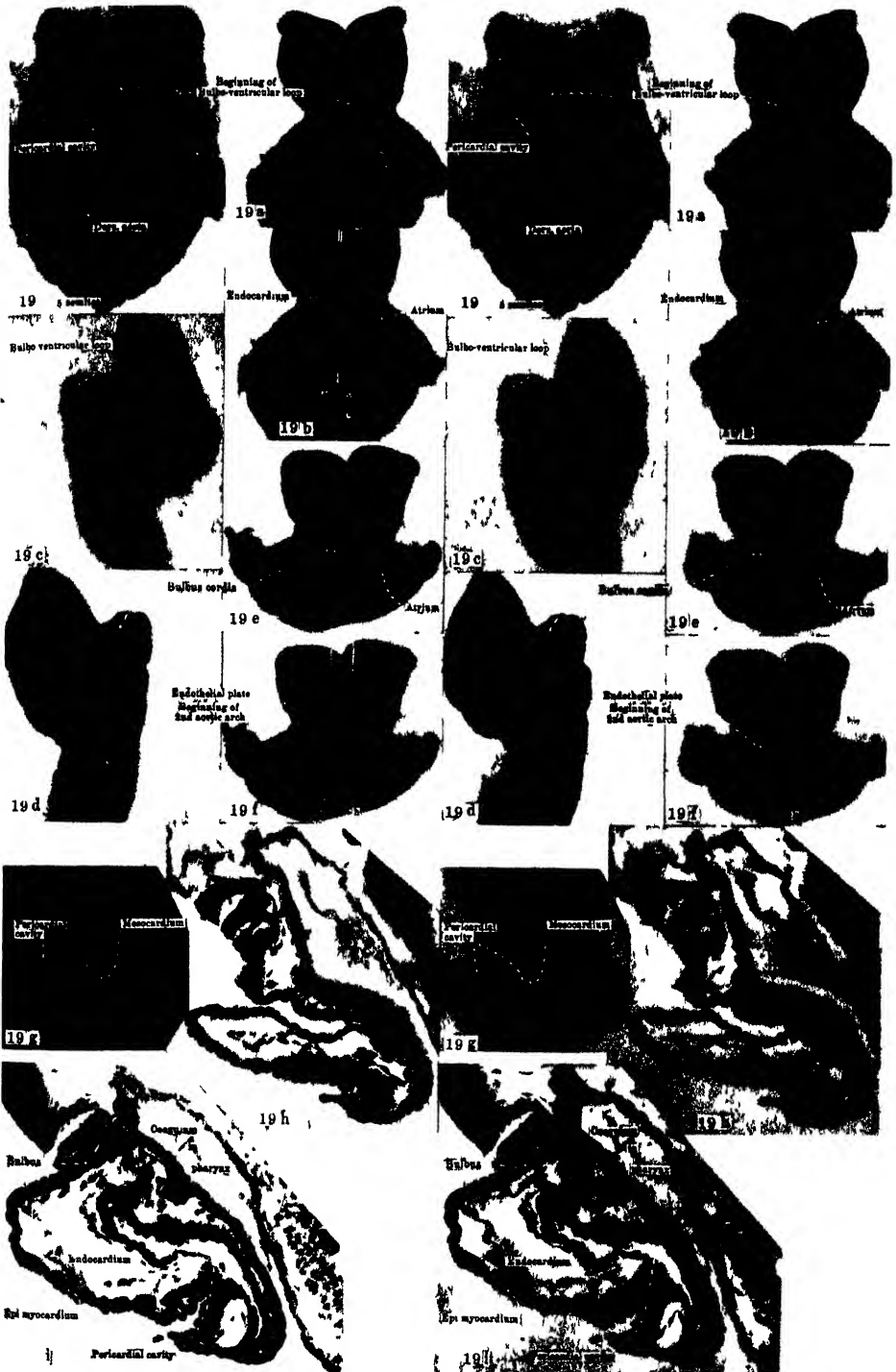


PLATE 34

Photographs $\times 35$, sections $\times 150$.
 $8\frac{1}{2}$ somites

Fig. 20. The heart displayed by the removal of the yolk sac, amnion, and most of the ventral body wall. The white lines mark the boundaries of the pericardial cavity and its posterior extension through narrow connections with the coelom. The mesocardium is very thin and extends along only part of the ventricular loop, so that the pericardial cavity is continuous above as well as below the heart. The atrial region is now clearly identifiable. The boundaries between bulbus and the ventricular parts are not conspicuous.

Figs. 20a, b, c. Different views of the same stage of dissection.

Figs. 20d, e, f. Different views with the ventral part of the myocardium cut away. In 20a and e are illustrated the endothelium of part of the ventricular loop, the bulbus, ventral aortae, and first aortic arch; also remnants of the endothelial plate, from which the second aortic arch seems to be in process of formation. In this $8\frac{1}{2}$ -somite specimen the second arch seems to be less advanced than in figure 18c. The endocardium of the rest of the loop, of the flat atrioventricular canal, and of the atrium is exposed in 20e and f. The extension of the atrial portions posteriorly and laterally may be considered the beginnings of the sinus venosus.

Figs. 20g, h. Sections nearly equidistant to the right and left of the median line. The photographs are unpaired. The myocardium is becoming thicker. The irregular surface of the endocardium seen in 20e and f is shown in the sections. This is the first embryo to show blood cells in the heart and vessels.

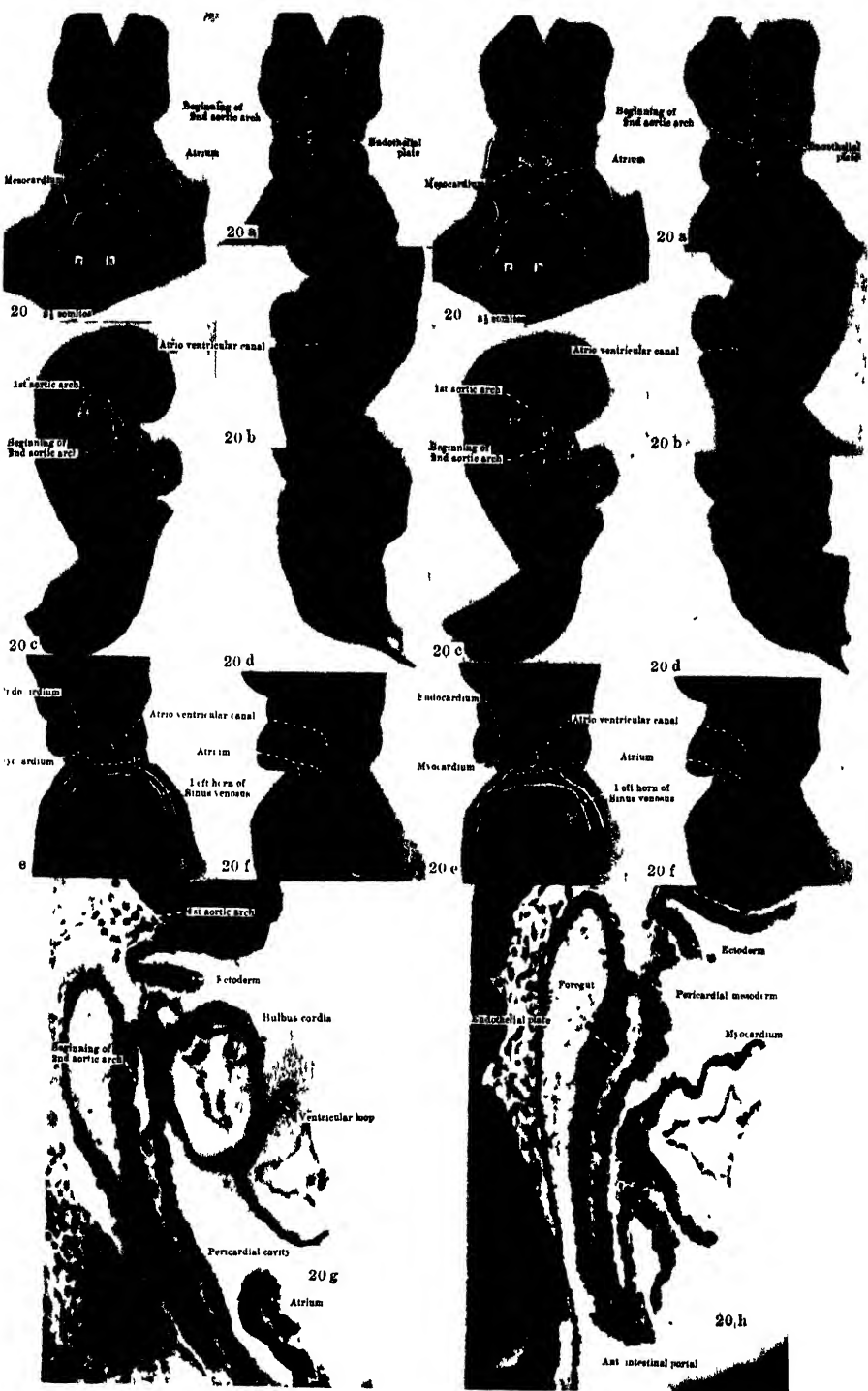


PLATE 35

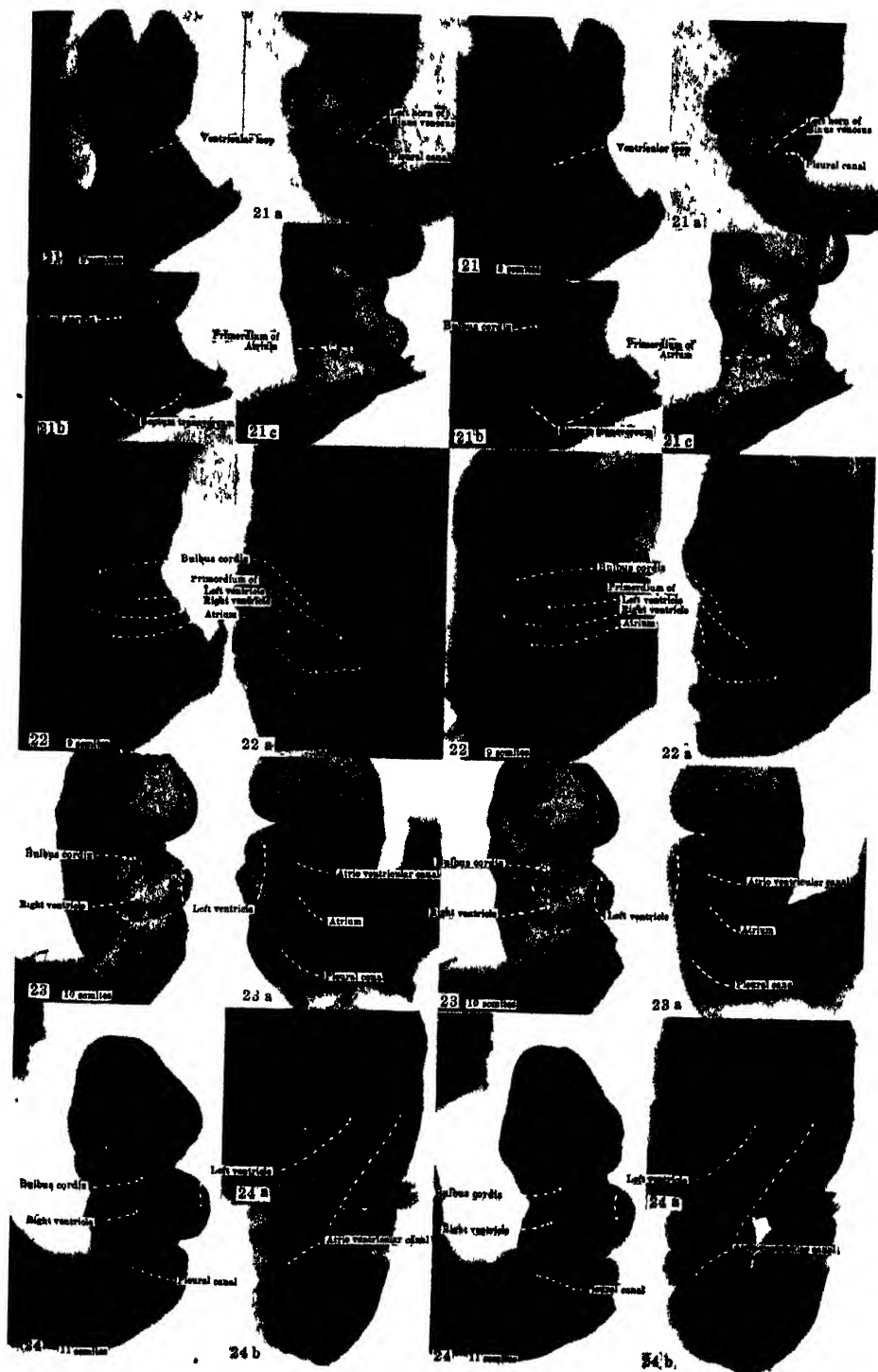
In this plate four embryos are so placed as to show chiefly the external aspects of the bulbus, the beginnings of the right and left ventricles, and the atrium. There is some variation in the relative sizes of the bulbus and parts of the ventricular loop which are probably not to be referred to different states of contraction at the time of fixing, but (as in earlier stages) are, more likely, slight variations.

Figs. 21, 21*a*, *b*, *c*. $\times 35$. Different views of the same dissection, except that in 21*a* the left side has been cut away a little more. The right side of the ventricular loop has been raised a little in order to expose the fundament of the right side of the atrium. In 21*a*, where the venous portion of the heart passes laterally the body cavity is constricted to form the pleural canals.

Figs. 22, 22*a*. $\times 35$. Two views of the same dissection. The ventricular loop has been raised slightly to show the atrium, but without obscuring the constrictions in the bulboventricular loop, in which may be distinguished the bulbus, very small right ventricle, and large left ventricle.

Figs. 23, 23*a*. $\times 35$. The right ventricle as compared with the left is larger than in figure 22.

Figs. 24, 24*a*. $\times 20$. Fig. 24*b*. $\times 15$. Very small right, and large left, ventricular regions. Because of the torsion in the body of the embryo the right pleural canal is less compressed than the left, which in this specimen has been cut open.



PLATES 36, 37, 38

In the next three plates are offered photographs of hearts that have been injected with ink and cleared in creosote to facilitate demonstration of the endothelial structures. The 8-somite stage is the youngest that can be injected, for in it circulation is just beginning, as shown by the presence of blood cells (figs. 20*g*, *h*). It will be seen that these injected specimens agree with results obtained from sections displayed in the preceding plates. Thus, the beginnings of the second, third, and fourth aortic arches seem to be associated with the endothelial plate, or its derivatives, although naturally the plate would not become visible as the result of injections. Nevertheless, in figures 25, 27, 28, 29, 31, and 32 it looks as if ink had made its way into the sprouts growing posteriorly from the aortic sac. Attention may also be directed here to certain characteristics common to the figures just named. The endothelium in the bulbus is flat and twisted, a torsion that may well be the forerunner of the spiral nature of the future bulbar septum. The atrioventricular canal is also much flattened in a dorsoventral direction; likewise the atrium, at least in the early stages. At the venous end of the heart the vitelline, umbilical, and common cardinal veins on each side connect with the atrium through a single short trunk that may be considered the beginning of the sinus venosus and called the horn of the sinus. The point or area of union is variable and is such that it cannot be said that any vessel opens into another.

PLATE 36

Figs. 25, 25a. $\times 25$. From the right side and ventral aspect, respectively. Well-formed ventricular loop. The atrium is little more than a continuation of the horns of the sinus into the atrioventricular canal.

Figs. 26, 26a, b, c. $\times 35$. Four stages of dissection. The atrial region of the myocardium, visible in the earlier stages as slight elevations, is well marked and shows distinct right and left enlargements. The atrioventricular canal opens into the left.

Figs. 27, 27a. $\times 35$. Two views of the same. The endocardium corresponds perfectly to both the endocardium and the myocardium in figure 26.

Fig. 28. $\times 35$. (Also fig. 28a, pl. 37.) The chief advance is in the atrium, which is longer in an anteroposterior direction. It is broadly continuous with the two horns of the sinus.

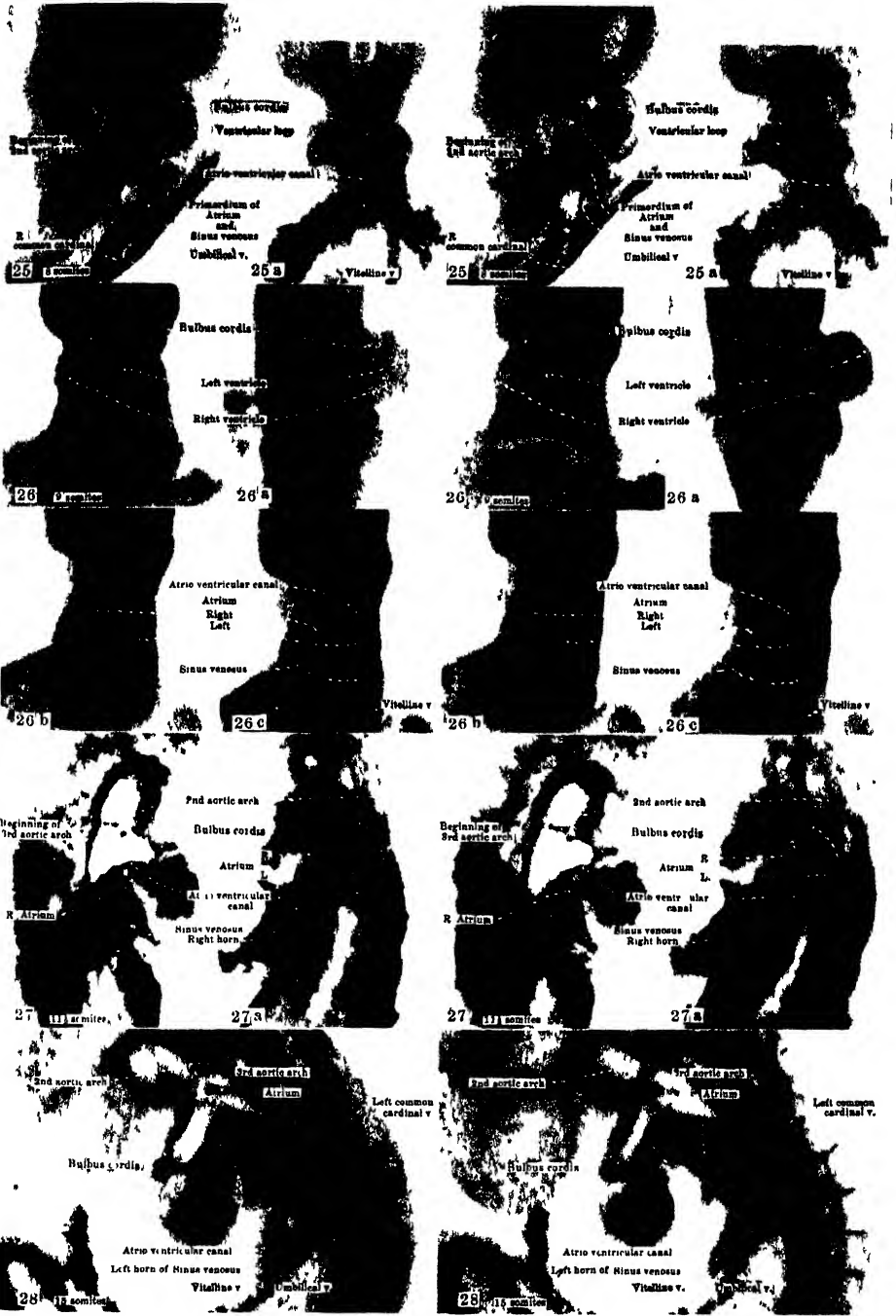


PLATE 37

Fig. 28*a*. $\times 35$. See plate 36. Dorsal view.

Figs. 29, 29*a*. $\times 35$. Figure 29 is viewed from the left side, and figure 29*a* with the loop removed at the atrioventricular canal from the ventral. Right and left atria well marked as anterior prolongations of a median portion, which is narrowed posteriorly to receive the sinus horns.

Figs. 30, 30*a*, *b*, *c*, *d*. $\times 30$. Five stages of dissection. Close correspondence with the embryos of figures 28 and 29. Although in the ventricular loop the right and left divisions are separated by a well-marked constriction, there is no internal septum at all.

Fig. 31. $\times 20$. Fig. 31*a*. $\times 30$. Viewed from the left side; and ventral side after removal of ventricular loop. Atrium deeper dorsoventrally. Sinus horns are shorter. The truncus arteriosus is the narrow terminal portion of the bulbus in which the endothelial lining is closer to the myocardial layer than in the rest of the bulbus. It lies within the limits of the pericardial cavity, which extends as far as the aortic sac which is the point of origin of the arches. The truncus may be identified, also, in figures 33*b* and 37*b*.



PLATE 38

Fig. 32. $\times 20$. Fig. 32a. $\times 30$. The comments on figure 31 apply to this stage. Through the stages of development illustrated by the injected embryos it will be observed that the atrium, at first posterior to the ventricular loop, as it grows and differentiates comes to be dorsal to the loop, while its right and left prolongations come to lie on the right and left sides of the bulbus. The atrium and ventricular loop maintain these relative positions with scarcely any change up to 15 and 16 days, when the atria are found anterior to the ventricles. Consequently, a transverse section through the body of the embryo is approximately a longitudinal section through the heart from right to left, or what for convenience may be called a frontal section. In frontal dissections of the heart the organ would be seen in posterior or anterior view.

Figs. 33, 33a, b. $\times 20$. Three stages of dissection in ventral view. The differences in the structures of the walls of the truncus, bulbus, and ventricle are easily discernible even though no external grooves mark the limits. The right ventricle is larger than in the earlier stages. It is demarcated from the left ventricle externally before the internal septum appears. The atrioventricular canal is no longer flat, but round, and opens into the left atrium.

Fig. 34. $\times 25$. Dissection from anterior side, the bulbus and part of the wall of the ventricular loop having been removed, so that the observer looks posteriorly into the sinus venosus. The two horns of the latter unite immediately before opening a little to the right of the median line. An interventricular septum is in the earliest stage of formation. As in figures 33b and 35, the atrioventricular canal opens into the left ventricle.

Fig. 35. $\times 25$. Similar to figure 34. The opening of the left horn is moving to the right side of the atrium as though slipping past the primary interatrial septum (which is at its inception) to open into the right horn.

Fig. 35a. $\times 25$. After removal of the ventricles and most of the atrium wall.

Fig. 36. $\times 25$. (Continued on pl. 39.) The heart exposed from the right side with the right auricle, right horn of the sinus, and its tributaries opened.

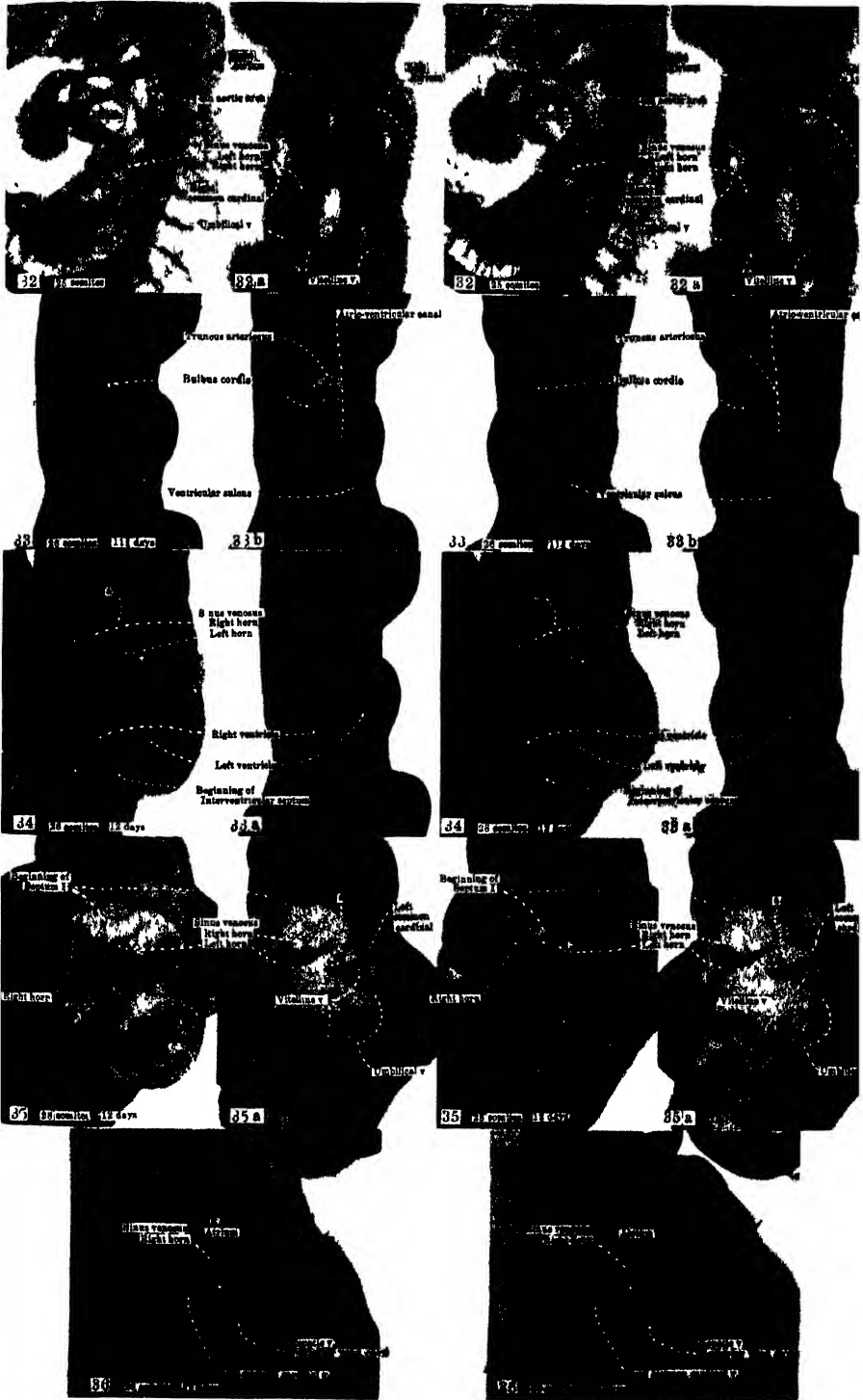


PLATE 39

Fig. 36a. $\times 25$. A further stage in dissection from the right side, showing particularly the opening of the left horn of the sinus into the right horn.

Figs. 37, 37a, b. $\times 25$. Three stages in lateral dissection to show the beginnings of the interatrial septum, interventricular septum, the atrioventricular canal, and the veins on the left side. In this specimen the left horn of the sinus still opens well to the left in the atrium. As in figures 34 and 35a; there is at *a* a small mass of nodular growth associated with the primary septum, of unknown significance. It may be followed in later stages in figures 38a, b, 41, 43a, 44b, 53c. Similar nodules occur elsewhere, as on the cusps of the sinus valve (figs. 41, 57).

Fig. 38. $\times 20$. Left exterior. See further details on plate 40.

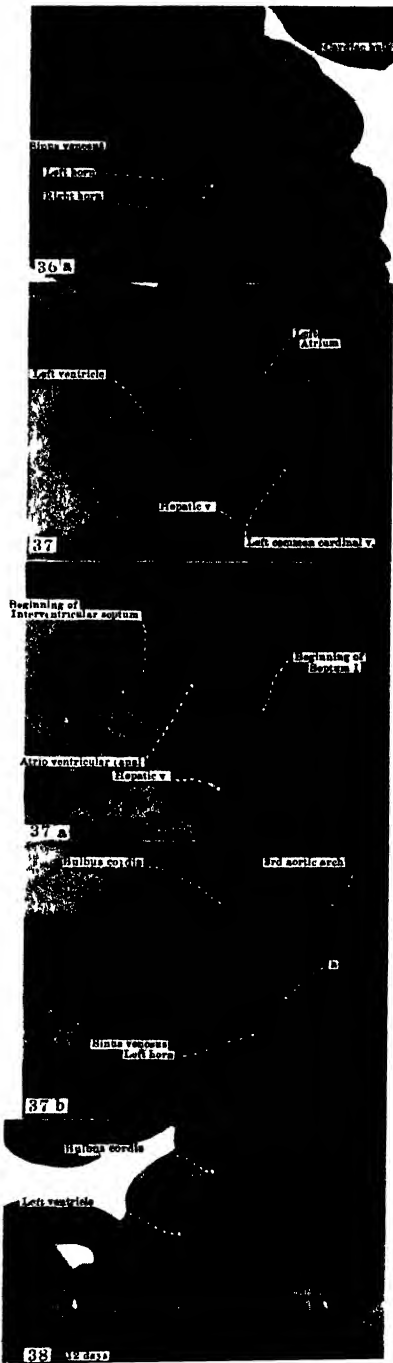
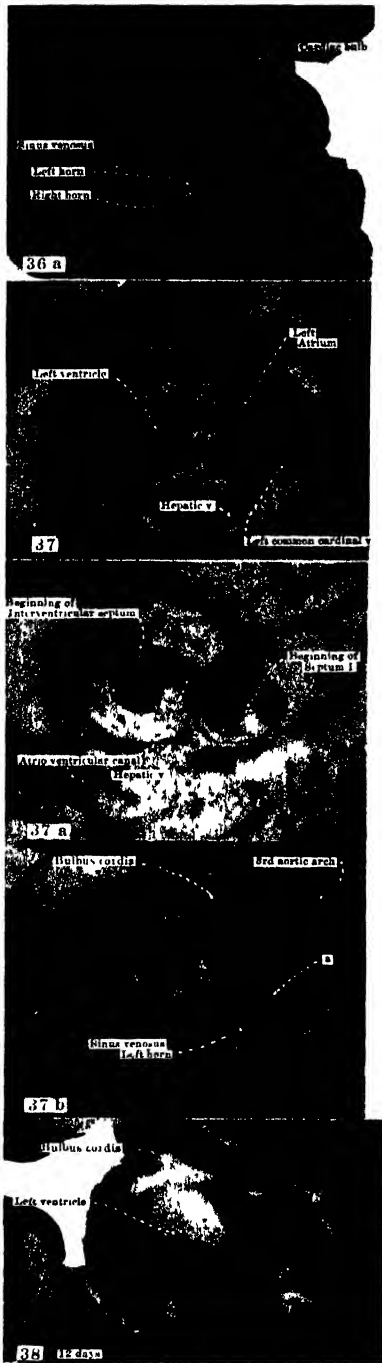


PLATE 40

Figs. 38*a, b*. $\times 20$. Further stages of dissection of heart shown in figure 38. Septum 1 is broader and already shows signs of becoming thin where the foramen is to appear. Its margin is slightly thickened. Endocardial cushions are beginning to form in the atrioventricular canal.

Figs. 39, 39*a, b*. $\times 25$. Three ventral views of transverse dissections of the heart; ventricles removed. The spirally coursing bulbar septum cushions are forming partly as the result of infiltration of cells between endocardium and myocardium. Compare with the bulbus in figures 37*b* and 38*b*. The bulbus clearly is connected directly with the right ventricle. Four atrioventricular cushions are formed.

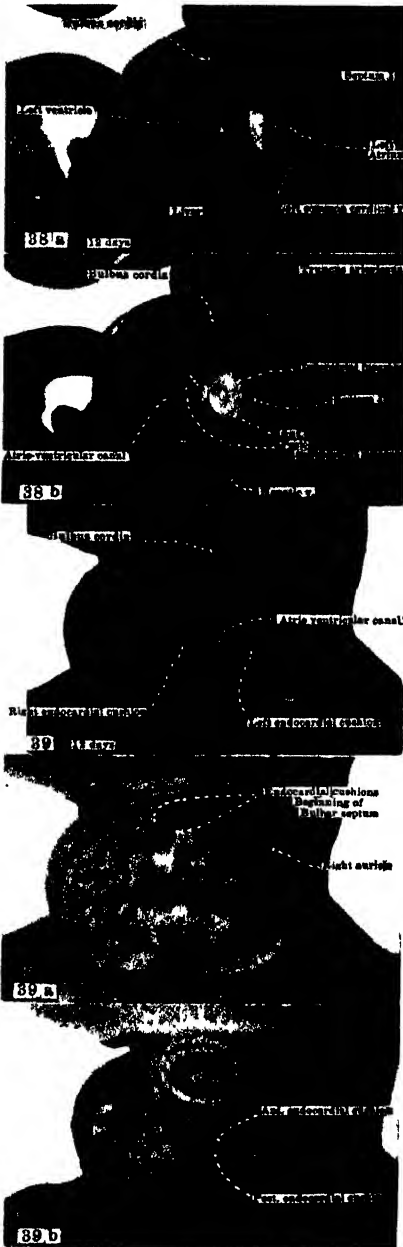
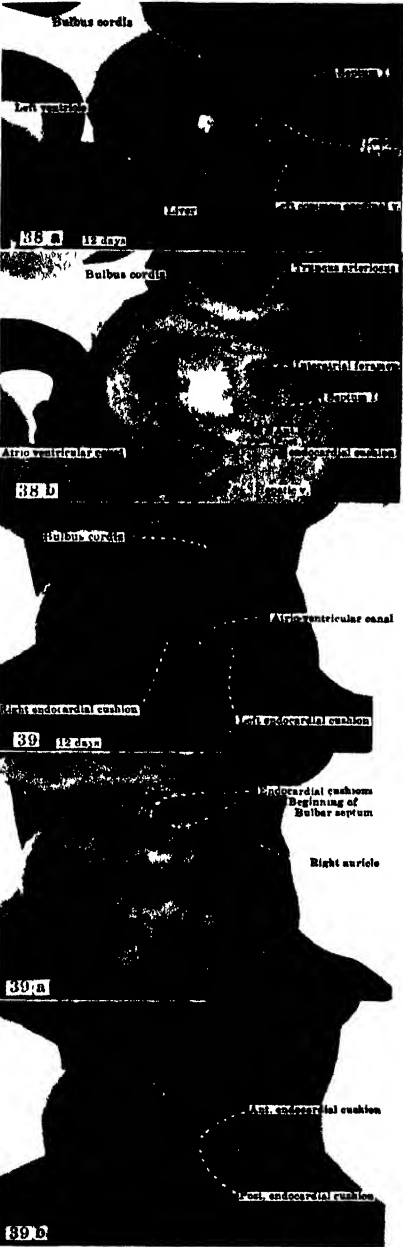


PLATE 41

Fig. 40. $\times 15$. Dissection from right side. Septum 1, which in figures 34, 35, and 37 is well to the right of the atrioventricular canal, in figure 38*b* and in this figure comes to be directed toward the middle of the canal, where it remains in later stages (fig. 41, etc.).

Figs. 41, 41*a*. $\times 20$. Dissections from anterior; bulbus, et cetera, removed. The opening of the sinus has shifted to the right atrium. It shows a slight constriction, which may be interpreted as persistence of the separate openings of the two horns of the sinus, after the migration of the left horn toward the right side. The secondary interatrial foramen, though not clearly shown, is nevertheless evident.

Fig. 42. $\times 20$. Ventral dissection; ventricles removed. Further advance in development of the bulbar septum and canal cushions.

Figs. 43, 43*a*. $\times 10$ and $\times 18$. From the left side. The mode of origin of the secondary interatrial foramen clearly shown. With the growth of septum 1 and foramen 11, foramen 1 diminishes.

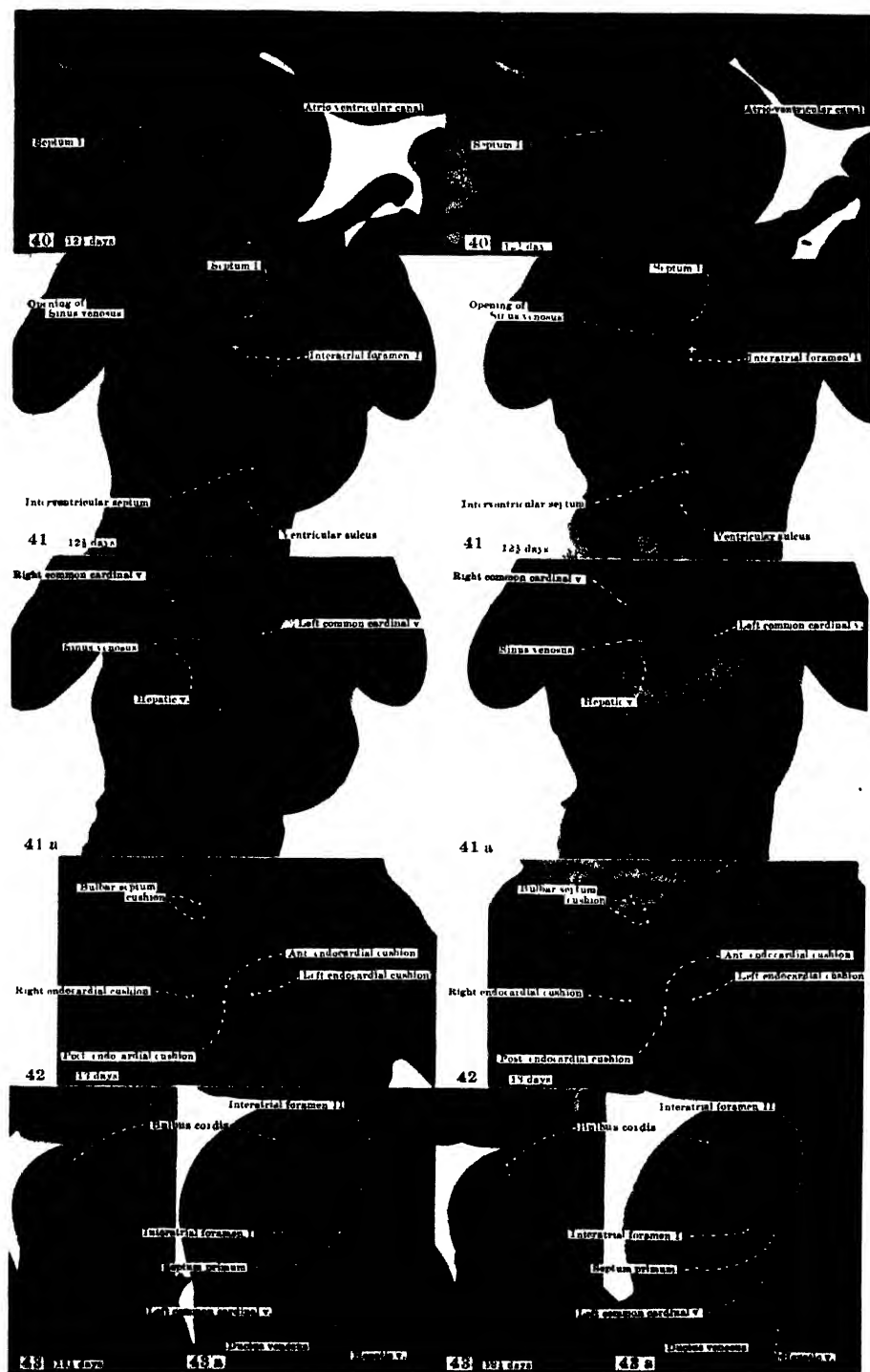


PLATE 42

Figs. 44, 44a, b, c. $\times 20$. Four stages of anterior dissection. Orifice of sinus is now single, the two horns or common cardinals uniting just outside the orifice. Sinus valve cusps appearing. These are united at their dorsal ends in the so-called septum spurium. Foramen II arising from several apertures. First appearance of the pulmonary vein.

Fig. 45. $\times 15$. On the right side the veins and the right atrium are opened, leaving the free edge of the false septum in position. The orifice of the left common cardinal into the sinus is somewhat restricted. Comparison with the two preceding figures shows that it lies under the proximal portion of the right cusp of the sinus valve, or what may be considered an extension of that cusp: a point to be kept in mind when the later stages are studied.

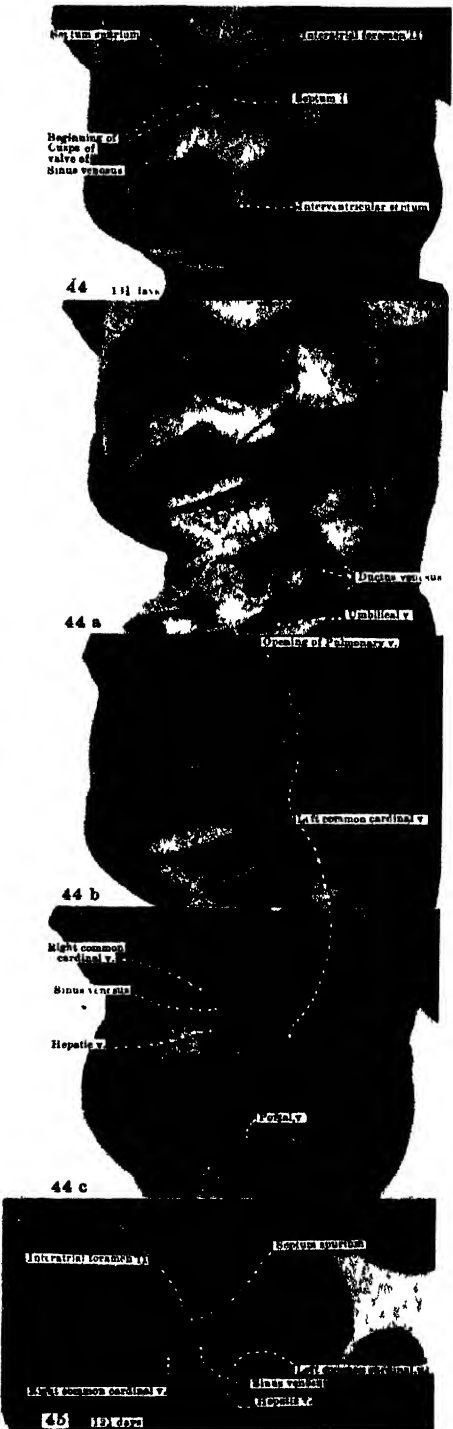


PLATE 43

Figs. 46, 46a. $\times 25$. From the right. In figure 46 the two dotted lines show the level at which the valve cusps, et cetera, were cut to get the dissection in 46a. Valves wide open. Orifice of left common cardinal much restricted. It is directed toward the opening of the sinus into the atrium, and lies more definitely at the base of the right cusp, which, in the closed condition of the sinus valve in figure 47, effectively covers it (compare with fig. 48). The advancing edges of the septa in the atrium, ventricle, and bulbus are thick and rounded. The atrioventricular canal and its cushions are undivided. Indeed, they do not divide independently, but in association with the arrival of the interatrial septum and its union with the cushions (see figs. 51a, 53). The truncus arteriosus is already completely divided to form the distal portions of the systemic and pulmonary trunks, the proximal portions of the two trunks being continuous with the right ventricle (to the right of the interventricular septum).

Fig. 47. $\times 20$. Showing the sinus valves and opening of left common cardinal closed. The atrioventricular valve cushions are nearly in contact, and the cusps of the sinus valve are as noted above. The division of the truncus is more clearly displayed, and the continuity of one of the bulbus cushions with the interventricular septum may be seen. The interatrial septum is about to unite with the atrioventricular canal cushions.

Fig. 48. $\times 20$. Similar to figures 46 and 47.

Figs. 49, 49a. $\times 25$. Left and right views of the same dissection to show the atrioventricular valve closed. The two interatrial foramina seem to be open. Septum I protrudes into the left atrium.

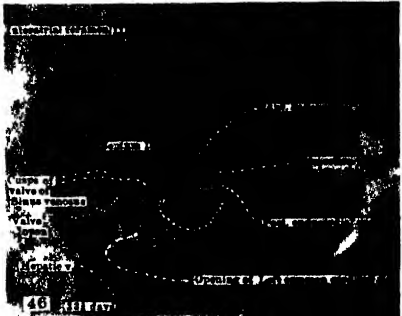
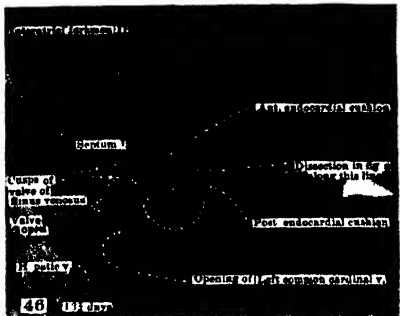


PLATE 44

Figs. 50, 50*a*. $\times 20$. Two stages in ventral dissection of the atrioventricular canal and bulbus. The lower ends of the bulbar septum cushions have not yet united. One of the cushions is continuous with the right wall of the right ventricle just below the right endocardial cushion of the atrioventricular canal, and the other with the interventricular septum, which latter in turn unites with the posterior endocardial cushion. These are to be compared with plates 48 and 49.

Figs. 51, 51*a*, *b*. $\times 10$ and $\times 17$. Three stages in dissection from the anterior (and ventral side). Externally the bulbus is not divided. Septum primum has reached and united with the atrioventricular canal cushions so that the latter now constitute two separate valves. As evident in figure 44*a*, the lower ends of the sinus valve cusps tend to unite with the right side of septum I near its edge and, at this stage, with the posterior endocardial cushion, bringing the opening of the sinus valve close above the right atrioventricular valve. Understanding of this helps to clarify the figures in plate 49. In figure 51*b* a portion of the right cusp of the sinus valve is cut and turned back, revealing again how the left common cardinal opens under it. Into the same venous sinus open also the right common cardinal and the hepatic vein. The latter vein has been so labeled up to this time because it has been the efferent vessel of the liver only. However, after the posterior vena cava has been established and as it develops as a major trunk through the liver, receiving the liver efferent vessels, it may be thought of as taking over the original efferent trunk as its anterior constituent.

Fig. 52. (Figs. 52*a* and 52*b* are on the next plate.) $\times 20$. One of three slightly different views of one dissection from the dorsal side. In this specimen the interatrial septum has not yet fused with the atrioventricular cushions. The relations of the ends of the cusps of the sinus valve to the septum spurium and the anterior endocardial cushion are clearly shown; also the relations between the vessels entering the sinus, already discussed.

This heart is a little less advanced than that in figure 51. For another of the same age see figure 54, plate 46.

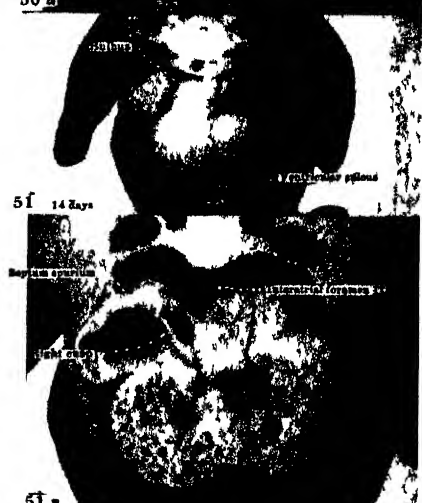
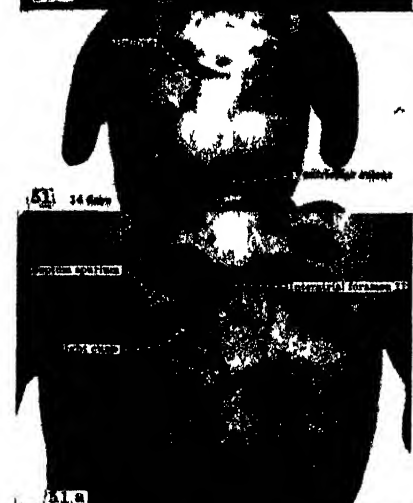
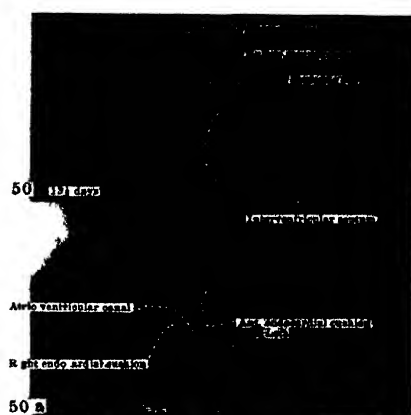
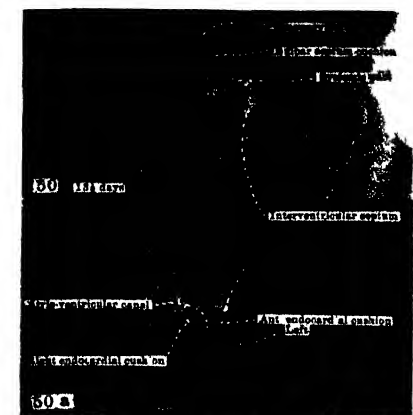


PLATE 45

Figs. 52*a, b*. See preceding plate.

PLATES 45, 46, 47

Figs. 53, 53*a-i*. Different stages of dissection from both sides, and different views with different backgrounds. The last two are left and right aspects at the last stage, in creosote. Figs. 53, 53*a, b*, and *i*. From the left side. Septum I has united with the endothelial cushions; its free edge is slightly thickened. The part marked "septum II" corresponds to what is so called in other animals. It is not a true septum (see text, p. 256). The bulbar cushions have not united proximal to the point where they contribute to the formation of the semilunar valves; one cushion unites with the interventricular septum. The opening of the left common cardinal is guarded, as discussed already, by the right cusp of the sinus valve. Figs. 53*c-h*. From the left side. The pulmonary vein orifice is close to septum I. Of the truncus arteriosus (which precedes the bulbus in division) the left half takes over the pulmonary arches. The orifice of the right pulmonary arch can be seen; the left arch, or ductus arteriosus between the pulmonary artery (fig. 53*g*) and the dorsal aorta, is beyond the limit of the dissection. The semilunar valves arise from the bulbar septum cushions and two other short endothelial cushions (figs. 53*h* and *i*, especially; also, figs. 42, 58*b*, and 59*d*).

In the bulbus and truncus the septum cushions follow the original spiral clearly shown in figure 32. Comparison of figures 32 and 53*h* is particularly instructive. Both sides still open into the right ventricle. These conditions may be followed further in the next two figures.

Fig. 55. $\times 12$. Ventral (anterior) dissection showing especially the bulbar septum cushions.

Fig. 56. $\times 20$. Much of the ventricle has been removed so as to expose the atrioventricular valves, which are now separate, and the partly divided bulbus.

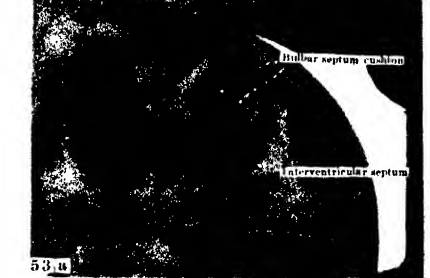
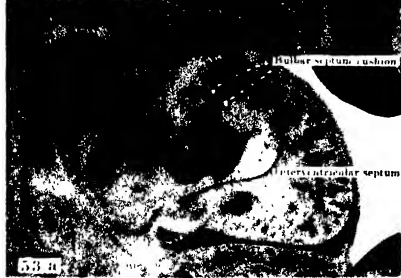
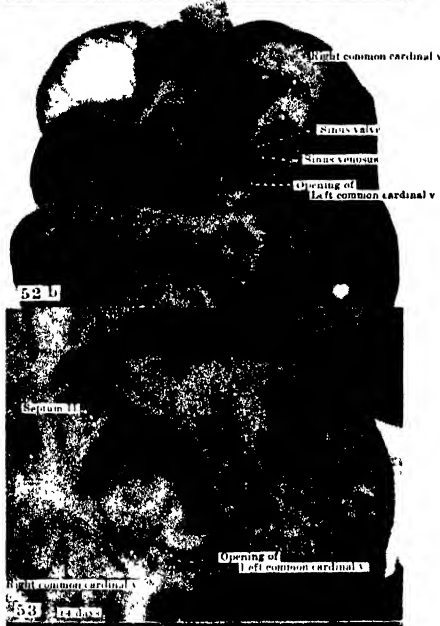
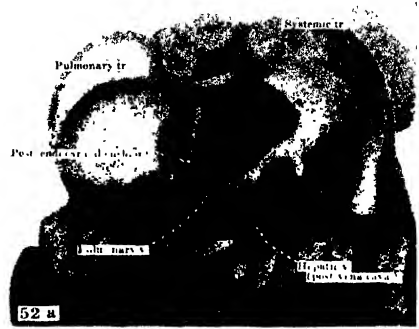
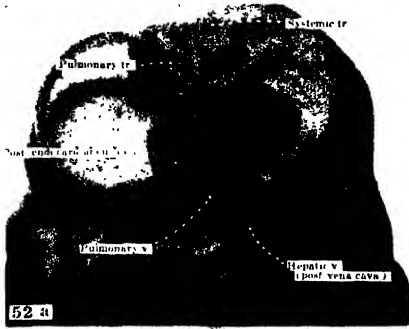


PLATE 46

Figs. 54, 54*a*. $\times 20$. These figures are placed here for economy of space. The embryo is of the same age as that in figure 52, and slightly younger than that in figure 51. In these two stages of dissection the relation of the opening of the left common cardinal to the sinus valve cusps is clearly shown. The right cusp is bent to the right in figure 54*a* after being cut along the lines marked in figure 54. For the significance of "b" see explanation of figure 57*a*, plate 48.

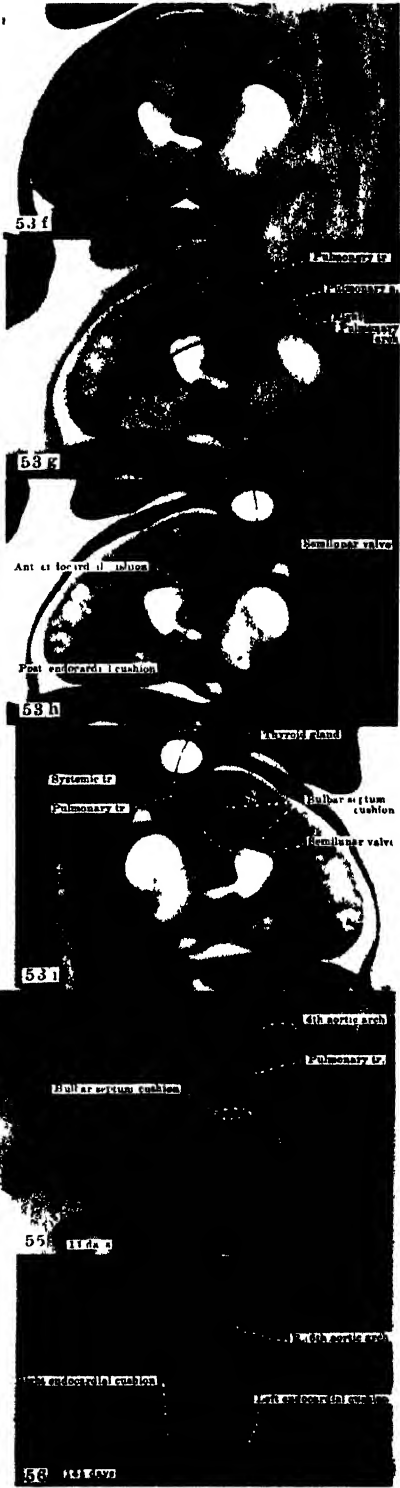
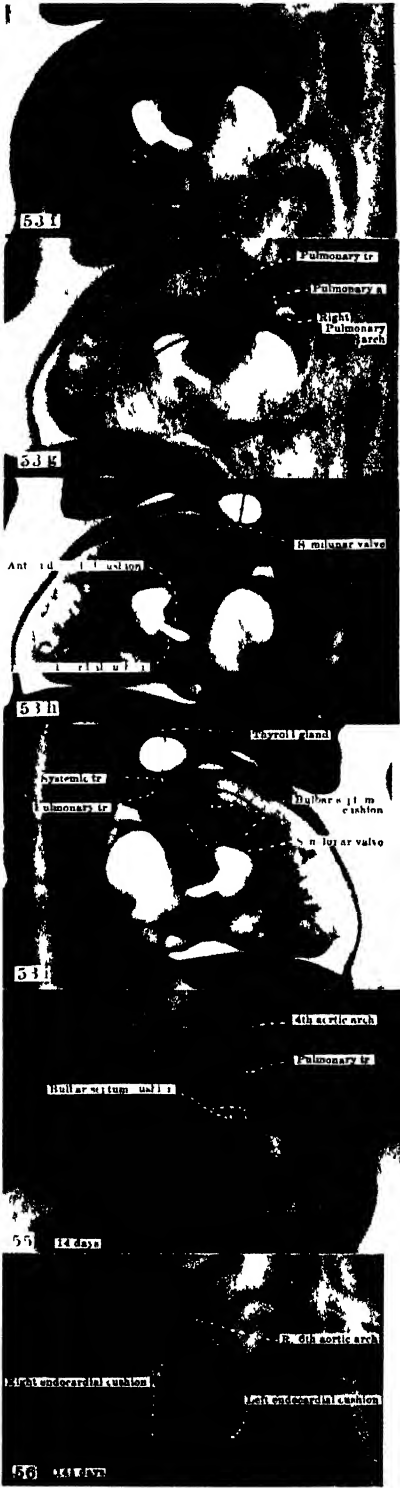


PLATE 47

For explanation of these figures see description opposite plate 45.

PLATE 48

Figs. 57, 57a. $\times 18$. Dissections from the right, intended to show the relation of the left common cardinal aperture to the right cusp of the sinus valve. There is now started a process by which the common cardinals or anterior venae cavae and the posterior cava come to open independently of each other to a considerable extent at least by the third day after birth. In figure 54a (pl. 46) a band of tissue marked *b* is seen to extend from about the point where the valve cusps reach the primary septum, along the edge of the opening of the left common cardinal and on to the wall of the posterior vena cava. It therefore passes between the bases of the two cusps. This same strand, marked *b*, scarcely detectable in figure 57a, is also labeled in figures 60a, 61d, and 62, in which it is clearly visible. By comparing these five figures it can be seen that as the right anterior and the posterior venae cavae seem to draw away from each other to the accompaniment of elongating sinus-valve cusps, this strand or band of tissue (labeled *b*) seems to rise between the left anterior vena cava and the sinus venosus (fig. 60a). With the continuation of these changes the left vena cava comes to open into the atrium instead of into the sinus, under an extension of the right cusp (figs. 61d and 62). The extensions of the cusp and the band of tissue together may be considered to act as a valve for the left vena cava, one thin, the other relatively thick.

Figs. 58, 58a, *b*. $\times 20$. Ventral (ventroposterior) dissections in three steps. Figs. 58a and *b* are two views of only slightly different dissections. In these three figures is depicted a stage shortly before the two ventricles are finally separated from each other and before they completely establish their separate connections with their respective parts of the bulbus. In the next plate these processes are nearly completed, and to understand the final stage it is helpful to see in figures 58–58b where the processes noted above are to take place. It should be remembered that the ventricles have been removed and that the halves of the bulbus open upward (in the photographs) into the ventricles.

At this stage the bulbar septum cushions are almost completely fused with each other, forming a thick partition. The left cushion is continuous with the interventricular septum, which in turn is already united with the posterior endocardial cushion of the atrioventricular canal, just to the right of its middle point. The right bulbar cushion passes into the right wall of the right ventricle. The interventricular foramen (fig. 58a, with the long arrow pointing into it) is still fairly wide. The pulmonary trunk opens only into the right ventricle; the systemic, while opening directly into the right ventricle, is at the same time in close communication with the left through the interventricular foramen.

With these details in mind it is easy to see the next steps to the final conditions which are indicated by the upwardly directed arrows in figure 58b. In the first place, the interventricular foramen does not close, but contributes to the permanent connection between the aorta and left ventricle. Secondly, the interventricular septum unites also with the anterior endocardial cushion, as shown by the short arrow attached to it in figure 58a. Lastly, the location of the final closing which separates the systemic trunk from the right ventricle is marked by the dotted line labeled \times in figure 58b, and unlabeled in the other two figures. This final closing thus involves complete fusion in concentric fashion of the interventricular septum, the tips of the two bulbar cushions, the right wall of the right ventricle, and part of the margin of the right atrioventricular valve. In figure 59 this last closing is almost complete, only a little orifice labeled "last opening to close" being left.

In the pulmonary trunk may be seen the short cushion, which, together with the ends of the long cushions, contributes to the formation of the semilunar valve. See the same in the next plate.

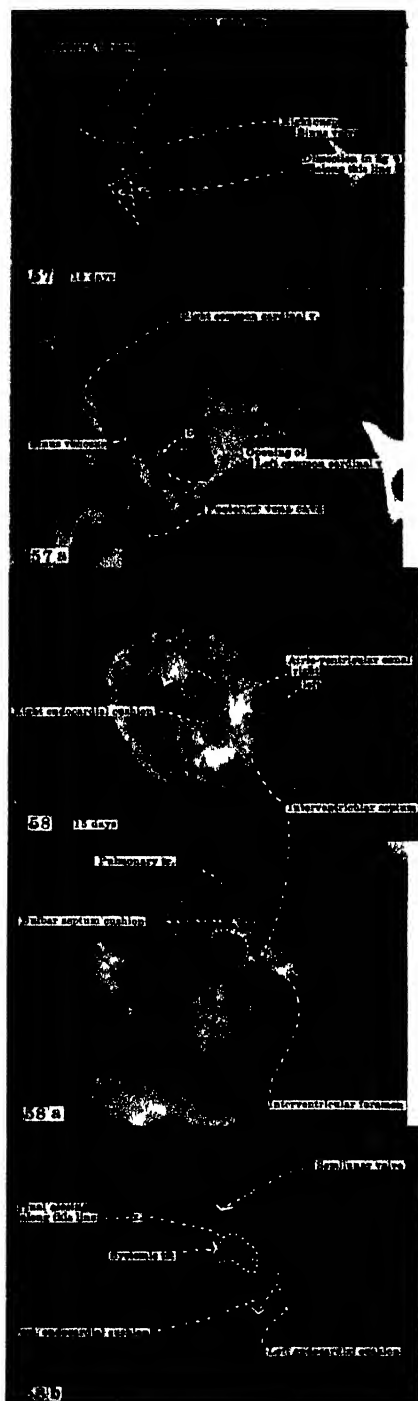


PLATE 49

Figs. 59, 59a-d. $\times 20$. Five photographs of as many slight advances in dissection. The ventricles have been removed except for their atrial ends.

In these figures are illustrated the processes described and figured on the preceding plate. The small orifice marked "last opening to close" is all that is left of the larger opening in figure 58 encircled by the dotted line labeled "final closing along this line." The approximate position of that dotted line in figure 58 is shown in figure 59b by a similar line marked \times . In these figures it is easy to identify all the steps of the processes to which attention was directed in the preceding plate. Thus the interventricular foramen, the connection of which with the right ventricle is soon to be severed at the "last opening to close," remains as the most proximal part of the systemic trunk. Since the interventricular septum united with the posterior (and anterior) endocardial cushion to the right of its center point, the right valve comes to lie closer to the wall between the two ventricles. These are the relationships found in late pregnancy (pl. 50, figs. 60a, c, d; pl. 51, figs. 61f, g) and after birth (plate 52).

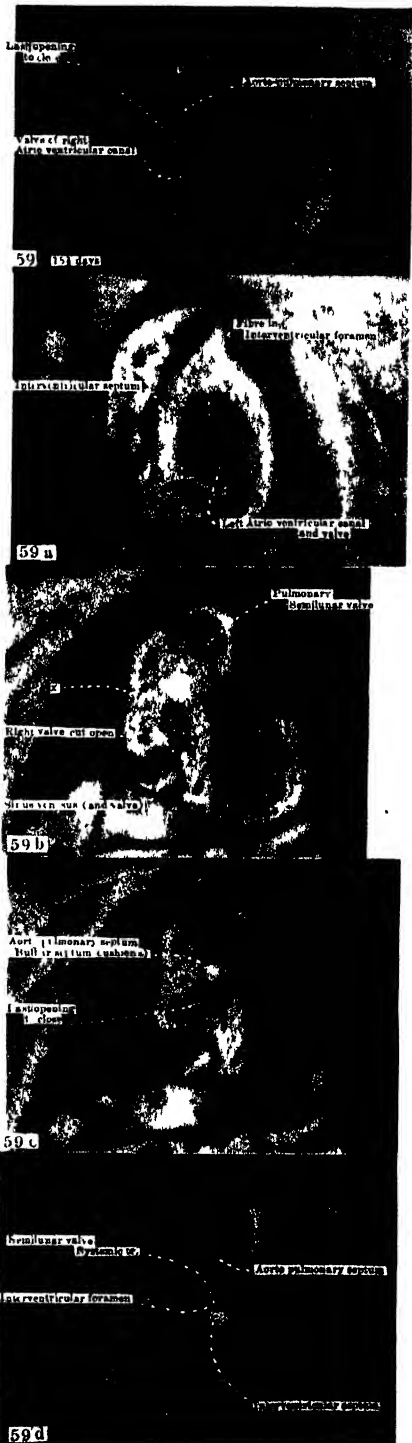
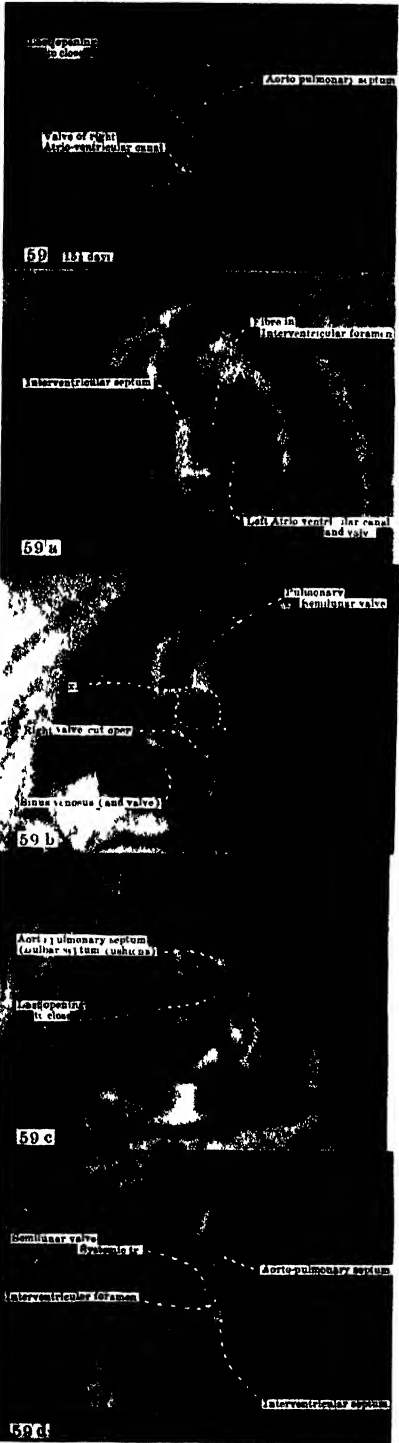


PLATE 50

Figs. 60, 60*a*–*d*. $\times 15$. Figures 60 and 60*a* from the right, *b* and *c* from the left, and *d* posterior-left lateral view of the upper ends of the ventricles. The first two figures show the sinus valve, the relation of the orifice of the left anterior vena cava to the right cusp, and the elevation of the tissue band “b” by which the left anterior vena cava comes to open separately into the right atrium, as discussed under plate 48. The detail in *d* shows the complete separation of the ventricles and the incorporation of the interventricular foramen into the systemic trunk. What is marked “septum II” is the narrow roof of the atrium at the bottom of a deep fold between the expanding atria. See text, page 257.

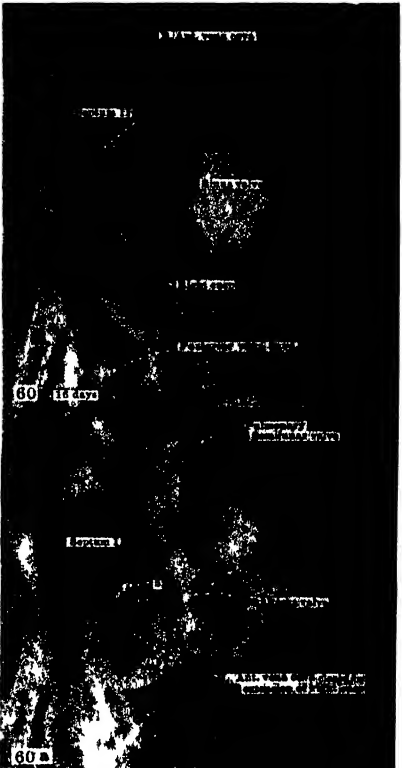
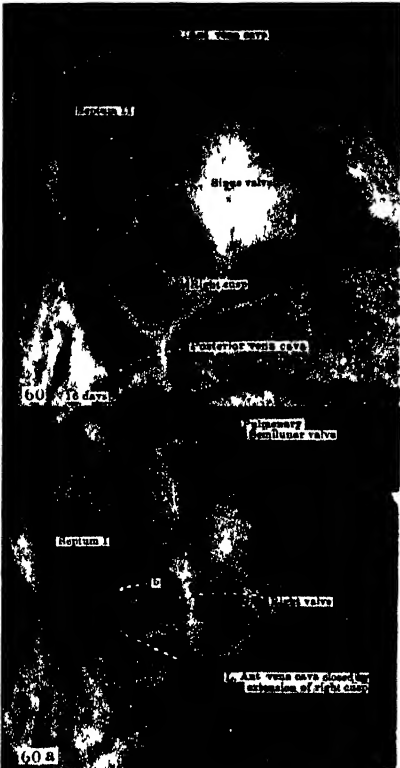


PLATE 51

Figs. 61, 61a-g. $\times 6$. The right anterior vena cava and the posterior vena cava open in common through a narrow slit between the two cusps of the "sinus valve," although a sinus venosus cannot be said to be present. The right cusp is extended (the Thebesian valve) to cover the orifice of the left anterior vena cava, the other margin of which is formed by the band of tissue "l" which comes to separate the left vena cava from the other two, as described under plate 23. Septum I forms a thin partition between the two atria. The development of the atrioventricular valve cusps with their chordae tendinae and muscles, well started in plate 50, has proceeded farther. The same is true of the semilunar valves. The nature of the walls of the atria and ventricles needs no comment. Arrows show the directions of movement of the blood, the blood which enters through the sinus valve being able, of course, to pass through the interatrial foramen also more directly than indicated.

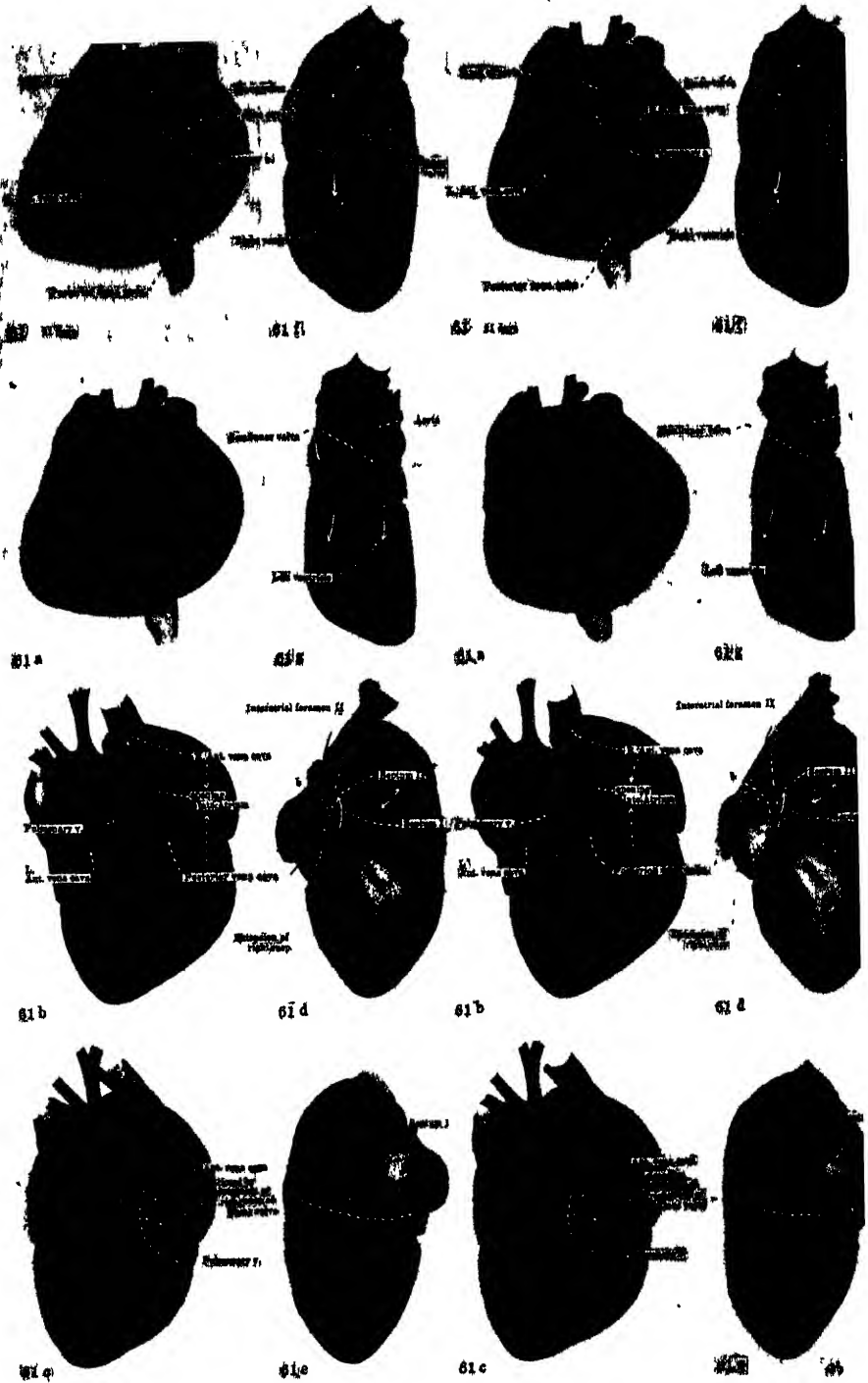
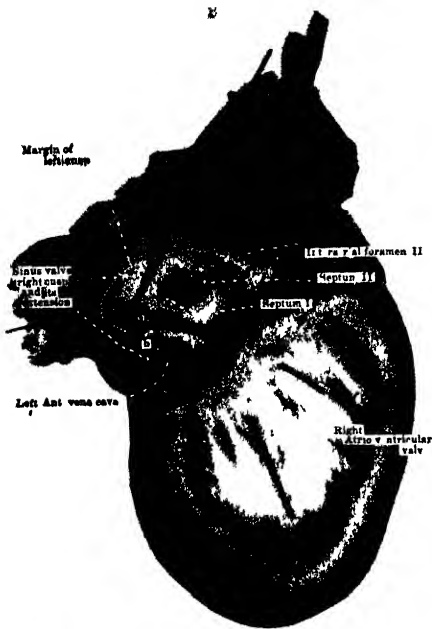
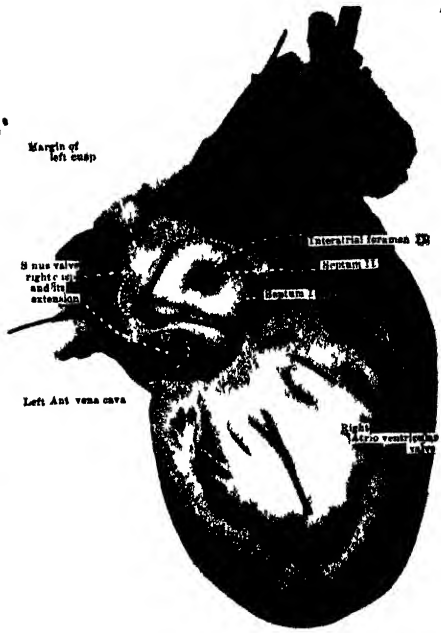


PLATE 52

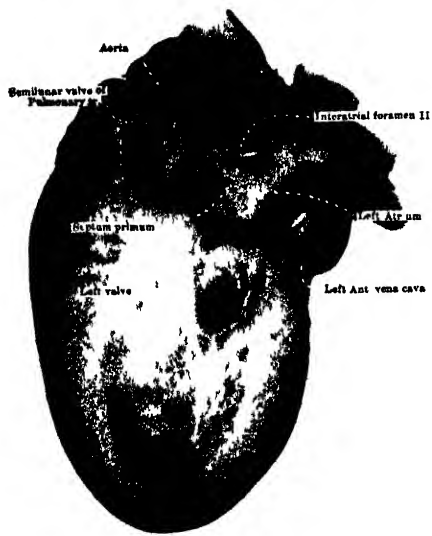
Figs. 62, 62a. $\times 10$. To show the method of closure of the secondary interatrial foramen, which is accomplished by fusion of the free edge of septum I from its ends toward its middle point, with the wall of the atrium. There is no true secondary septum. The orifice of the left vena cava, as already discussed, is flanked by the extension of the right cusp of the sinus valve (Thebesian valve) and the tissue strand "b."



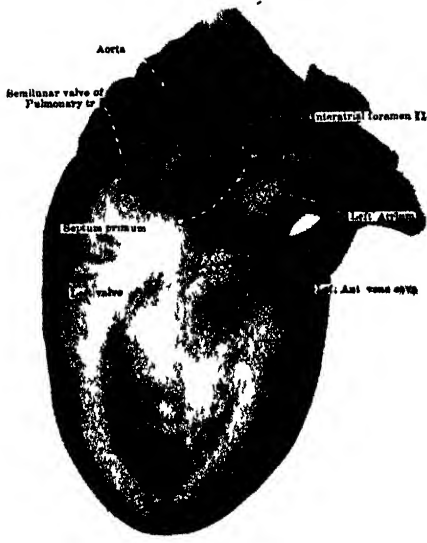
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**THE EARLY EMBRYOLOGY OF
TRITURUS TOROSUS**

BY

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AND

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INTRODUCTION

THE ANURAN AMPHIBIANS have long served as a valuable source of embryonic material. This is due, first, to their availability in many parts of the world and, secondly, to the fact that some of the early studies on their development became classic examples in embryology. Among some of the more important contributions may be mentioned the work of von Baer (1834) and Newport (1854) on the frog, and that of Schultze (1887) and the more recent work of Brachet (1902, 1927) on the Anura and Urodela. From the total of all contributions made on the anurans we have obtained a thoroughgoing knowledge of the descriptive embryology of this group.

The work of Wilhelm Roux (1885), also on the frog, introduced the method of experimentation, by which he attempted to ascertain certain causal relationships in development. This experimental procedure has been peculiarly effective in that it has served as a key to open up many problems. New and diverse methods of attack have been developed and extended to many types of animals. Within recent years, especially in the hands of Spemann and his students (1938), these procedures have been applied both to anurans and to urodeles, and have added much to our knowledge.

Notwithstanding the large use made of anuran material and the fact that certain phases of their development are unusually clear-cut, the anurans are more specialized than are the urodeles and their development reflects this specialization early. The development of the urodeles is therefore of great importance in giving a complete picture of development in the amphibian group.

Within the past few decades important studies on their development have been made in this country. Among these are the work of Jordan (1893) on the normal development of the egg of *Diemyctylus viridescens*, studies by Eycleshymer (1895) on *Amblystoma*, and a series of papers by B. G. Smith (1912, 1926) on the more specialized egg of *Cryptobranchus*.

With the appearance of Goodale's paper (1911) on the little-known form *Spelerpes*, an excellent account of the normal development was made available. In his paper, also, the method of vital staining was introduced, which demonstrated that great activity takes place in the egg during development. During the past decade this method has been extended and improved, largely through the work of Vogt (1929) in Germany.

Vogt's work warrants special mention. In his studies, which were made largely on the urodeles *Triton* and *Pleurodele*, he gave a good account of the normal development and, by the use of vital dyes, added a still more accurate and complete account of the movement of materials within the developing

egg. This work, coupled with that of Spemann and his students on the transplantation of parts from differently colored eggs of one species to the eggs of another, has furnished a method by which presumptive areas may be followed in their migrations to their definite positions.

The most abundant amphibian on the Pacific coast of North America is the urodele *Triturus*, and the eggs of the three species of *Triturus* (Twitty, 1936) are somewhat like those of the three species of *Triton*. In normal development the egg follows somewhat the same course, and many of the experimental procedures applicable to the eggs of *Triton* may be employed on the eggs of *Triturus*. In the present paper we give consideration to the normal development of *Triturus torosus*, beginning with the ovarian egg and following the development through to the laying down of the nervous system.* Experimental studies on the development of this form which the senior author has made during the past few years will be published later.

THE OVARIAN EGG

When the body cavity of a gravid female of *Triturus torosus* is opened, the ovaries appear as enormous masses filled with ova in different stages of development. It is an outstanding fact that these ovarian ova, although polarized, show no degree of response to gravity and consequently may have either the dark or the white pole upward while remaining in the ovary. At a stage of development somewhat later than that of the ovarian egg, however, the dark animal area of the egg is always directed upward, and the whitish vegetative area downward.

Within the ovary the ovum (fig. 1, pl. 53) is held within a surrounding follicle consisting of three layers of cells: an inner layer of follicular cells (*fc.*, fig. 2), a middle layer of cells composing the cyst membrane (*cm.*), and an outer layer which is the ovarian epithelium (*oe.*). Moreover, the individual ovum itself is surrounded by the zona pellucida (*zp.*), which becomes the chorion after the ovum leaves the ovary; and within the zona pellucida is the zona radiata (*zr.*), made up of fine alveoli, giving the appearance of radial striations in the surface of the egg.

Scattered pigment granules and yolk platelets extend throughout the whole of the cytoplasm (text fig. A), but the pigment granules are especially abundant in the superficial cytoplasm of the animal hemisphere. The yolk platelets are generally smaller around the periphery of the egg and, in this early ovum, are largest near the center (text fig. A and fig. 2, pl. 53), some of them extending well up into the animal area (fig. 5).

The germinal vesicle (*gv.*, figs. 1 and 3, pl. 53) lies within the animal half of the ovarian ovum and sometimes may be made out in surface view as a faint white spot. In sections it may or may not be surrounded by a space produced by shrinkage of the vesicle during fixation and imbedding. The vesicle early contains many nucleoli (figs. 1 and 3), among which are scattered granular threads of lightly staining chromatin.

* Assistance in the preparation of these materials was supplied by the personnel of Works Progress Administration Official Project No. 465-03-3-192.

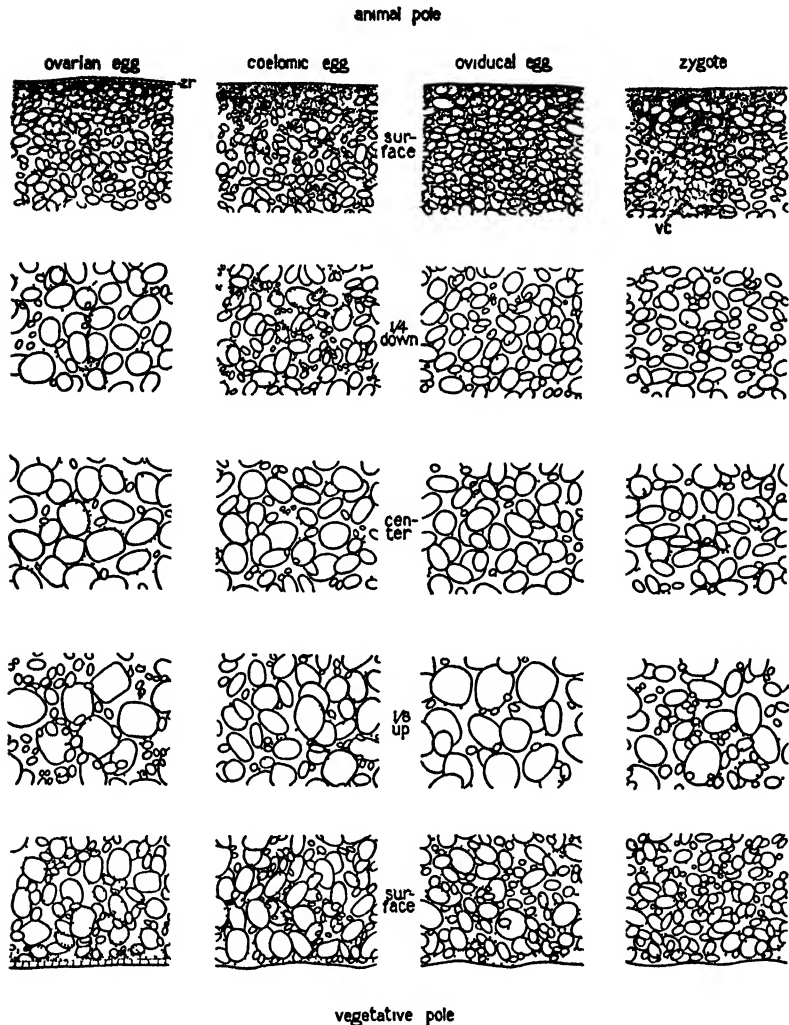


Fig. A. Camera lucida drawings through center of egg, showing distribution of pigment granules (as dots) and size of yolk platelets (in outline) in different regions of the ovarian, coelomic, and oviducal ova, and of the zygote of *Triturus torosus*. *vc*, vacuoles in animal area of zygote; *sr*, zona radiata. $\times 473$.

THE COELOMIC EGG

Eggs taken from the body cavity and studied in a watchglass containing a weak saline solution will rotate, displaying the marked contrast in the poles of the egg. The superficial pigment of the animal hemisphere, like that in figure 7, extends well down to the equator, separating this area from the vegetative region. The outline of the germinal vesicle soon becomes irregular, its wall ruptures (fig. 4), and the nucleoli (*ns.*) and the chromatin (*cr.*) draw together as individual masses. Soon the first maturation spindle appears (*sd.*¹, fig. 5), on which the chromatin is seen in the form of tetrads. The yolk plates

invade the space previously occupied by the germinal vesicle, and the large nucleoli are crowded out and come to lie near the periphery of the cytoplasm (*ns.*, fig. 6).

In sections, before the first polar body is formed, the cytoplasm over the spindle (fig. 6) appears as an area of the surface containing but few pigment granules, as contrasted with the rest of the surface of the animal area. Upon completion of the spindle, the first polar body (*pb.*¹, fig. 9) is extruded under the chorion, after which the germinal area will be readily visible in the living egg as a whitish circular area at the surface (*gr.*, fig. 7). The zona radiata that formed the inner surface layer of the ovarian egg has lost its alveoli in the coelomic egg and the egg is now bounded by an ordinary thin membrane. The zona pellucida remains as the chorion (see *ch.*, fig. 7) close to the surface of the egg until the egg shrinks; or it may be pulled away in sectioning.

After the completion and extrusion of the first polar body, and while the ovum is still in the coelom or is in the upper part of the oviduct, the second maturation spindle (*sd.*², fig. 10) forms with the chromosomes on it as paired V's and with its end against the germinal area. There is in the germinal area of entire eggs a tiny dark ring of pigment, the white center of which is the pole of the second spindle as it rests against the surface. The spindle retains this position until sperm entrance or, if the egg is unfertilized, until disintegration takes place.

THE OVIDUCAL EGG

When the ovum enters the oviduct it is devoid of all jelly layers. If we examine it after it has reached the lower third of the oviduct, however, we find that it has acquired all four jelly layers later present around the egg. These four layers at first are thin. Later they are seen to consist of a cloudy inner layer (see *j.*¹, figs. 7 and 8); outside of this inner layer of jelly is a thin irregular stratum (*j.*²), surrounding which is a tough striate layer (*j.*³); and encompassing all these is the outer clear layer (*j.*⁴). After contact with the water, regardless of whether the egg is fertilized or unfertilized (fig. 7), the first and fourth layers increase enormously in thickness.

THE EGG AT THE TIME OF LAYING

At the time of laying, the second maturation spindle (see *sd.*², fig. 10) may still have the chromosomes lying on the equatorial plate and one of its ends resting against the egg surface, with the longitudinal axis of the spindle perpendicular or tangential to the surface. If the egg is unfertilized, the spindle retains this state for hours until its final disintegration, but if a sperm enters the egg the second polar body early begins its formation. Frequently, at the time of laying, the second polar body is well along in the process of constriction, as in figure 11 (*pb.*²); or, if the egg has been retained for some time in the cloaca after sperm entrance, it may be essentially cut off from the egg (*pb.*², fig. 12), and the egg pronucleus may be nearing its time of formation.

Penetration of the sperm into the ovum takes place as we have indicated within the cloaca of the female, so that eggs which leave the cloaca are usually

found to be in the early stages of fertilization. It is difficult, however, to ascertain the actual time of sperm entrance into the egg, since eggs are retained in the cloaca different lengths of time after the deposition of the sperm cells on the jelly.

The early phases of sperm penetration may be well illustrated by figure 16, in which the head of the sperm has just perforated the surface of the egg and has passed a short distance into the cytoplasm. The thin surface layer of the egg around the entrance point of the sperm is elevated and vacuolated (*vn.*, fig. 16), and a pigment-free area of the cytoplasm extends inward as a cone around the head of the sperm cell. Surrounding this cone, and extending inward for a short distance along the head of the sperm, is an accumulation of pigment granules. The neck and tail (*tl.*) of the sperm still remain outside. In another egg (fig. 17)—which had been laid forty minutes—the whole sperm has advanced inward some distance from the surface of the egg. The sperm head is still compact and homogeneous in its staining; the neck occupies a light area surrounded by astral rays; and the tail lies in a yolk-free channel around which is an accumulation of pigment granules, the beginning of the so-called penetration path.

FORMATION AND UNION OF THE PRONUCLEI

After the second polar division occurs (figs. 13 and 14), the chromosomes of the egg break up and the cytoplasm above the chromatin material shows cross-striations (*cs.*). These cross-striations represent the remnant of the lower half of the spindle material as it now extends against the surface after elimination of the second polar body (*pb.*²). The surface soon smooths out and these striations disappear. A nuclear membrane is formed around the pronucleus (*pn.*, fig. 15), which rounds up and then moves toward the center of the egg, enlarging and becoming less dense as it passes inward. Whether it moves radially from the surface or in the direction of the sperm has not been determined, since the germinal area shifts its position somewhat, and the path of the egg pronucleus is not marked by a trail of pigment, as is that of the sperm.

In the formation of the sperm pronucleus the sperm head becomes vacuolated and increases in size, and the aster around the neck assumes prominent proportions (*ar.*, fig. 18). If the direction of the path taken by the entering sperm is away from the position of the egg pronucleus, the sperm head turns to move in that general direction. Early in its metamorphosis, however, the head of the spermatozoön ceases to move with the pointed end forward, as it entered, and the aster (*ar.*) and neck rotate so as to lie at the advancing end of the path, with the head dangling passively in the cytoplasm. As it trails inward following the neck and aster, the head enlarges, rounds out, becoming more vacuolated, and, gradually assuming the form of the sperm pronucleus (*♂*, fig. 19), it approaches the egg pronucleus. Figures 19 and 21 show the irregular path of the sperm pronucleus after the tail has disappeared and the head has largely lost its elongate shape.

As the sperm and egg pronuclei approach each other, long fibers having the

appearance of astral rays (fig. 21) extend ahead of and behind the sperm pronucleus in line with the latter part of the copulation path. It is difficult to ascertain just how completely the two pronuclei fuse together, but the examples available to us illustrating this phase of development show that the membranes in juxtaposition break down.

THE COMPLETED ZYGOTE

During fertilization other important changes take place in the jelly envelopes surrounding the zygote and in the zygote itself. As the jelly comes into contact with the water after the egg leaves the cloaca, or when it is brought into contact experimentally as in figure 7, the clear, sticky outer layer of jelly (*j.*⁴, figs. 7 and 8) begins to absorb water and to enlarge; and the inner, murky jelly layer (*j.*¹) shortly begins to swell and in the laid eggs to clear and to liquefy (Daniel, 1937). Moreover, during the liquefaction of the inner jelly two other changes take place within the zygote. The first of these is the formation, near the periphery of the animal area, of spaces or vacuoles (*vc.*, text fig. A) which are free from yolk platelets and pigment, and which may be traced into cleavage stages (text fig. E). The second change concerns the yolk. In the upper half of the animal hemisphere the platelets are small, and in the lower half of the animal hemisphere the plates break down into small platelets which, upon movement of the zygote, readily flow in the cytoplasm. Some of the central large plates of the ovarian egg (text fig. A) are broken up somewhat in the zygote stage, but others still remain as larger plates below the horizon. At this stage the vegetative hemisphere also becomes compact and rigid. Upon these changes within the cytoplasm of the egg, and upon the liquefaction of the inner layer of jelly, the zygote is entirely free to rotate and to settle. As a result the animal pole turns upward and the now heavier yoke-laden vegetative pole downward. At the same time, the egg which has been laid sinks down through the liquid inner layer of jelly and comes to rest on the floor of the second jelly layer.

Considerable movement in the animal hemisphere takes place during this primary rotation of the egg. The cytoplasm bearing superficial pigment and somewhat deeper yolk granules flows away from the area that will become the gray crescent side of the egg (see *gc.*, text fig. E), carrying the germinal area (*gr.*, fig. 7) for a short distance with it. A large amount of the superficial pigment collects on the side opposite the gray crescent to form the black shield (*bs.*, text fig. E). After these movements have been completed, the zygote appears to be tilted with the pigment horizon sloping obliquely upward toward the future gray crescent. Deeper movements also occur in the yolk granules of the animal area as these flow in the same direction as the pigment, leaving the top of the egg somewhat flattened as a superior plateau over which the chorionic arch rises (see *ch.*, fig. 7).

With these changes the zygotic pattern may be regarded as completed. The outstanding superficial feature of this pattern is the gray crescent (*gc.*, text figs. E-G), which carries over into the embryonic pattern as the dorsal organs;

the germinal area (*gr.* fig. 7) later occupies a position like a breastplate in the future embryo; and the black shield (*bs.*, text figs. E and F) comes to lie under the embryo and is associated with the ventral organs.

THE CLEAVAGE PATTERN

The first cleavage plane in *Triturus torosus* may pass along the sagittal plane of the future embryo, as it does in a relatively large proportion of anuran eggs, but this is far from universal, for, more frequently than in the Anura, it may take any position up to one at right angles to the future sagittal plane.

Preceding the first indication of surface cleavage in the egg there is produced internally (text fig. B) a faint line free from pigment and yolk granules which extends through the equator of the spindle and out toward the periphery of the egg, marking the future cleavage plane. In telophase (text fig. C) the surface of the egg shows an accumulation of pigment and a slight indentation at the point where the line through the equator of the spindle comes in contact with it. Under the surface depression below the cortex of the animal pole the vacuolated layer of cytoplasm takes a more decided dip than that at the surface, suggesting movement of this layer downward toward the spindle where the furrow will form. At a slightly later stage small isolated patches of pigment occur along this line as if the downward movement of cytoplasm had caught some of the surface pigment and carried it along with it. At this later time the movement of materials downward has filled the region between the surface and the spindle with dense cytoplasm in which the yolk granules are found to be finer than are the platelets below the spindle (text fig. D). Although there is a definite difference in density of the cytoplasm and the size of the yolk granules in the animal and vegetative areas before division begins, there is not the sudden transition at that time that is here observable.

Superficially the first cleavage furrow is indicated by a double band of pigment at or near the animal pole of the egg. This band soon deepens with the furrow and begins to extend vertically farther down the egg. In the meantime the cytoplasm of the animal pole on both sides of the furrow becomes elevated into hummocks (*hm.*, fig. 22), with each hummock tapering off at the sides but descending steeply into the furrow. In the first part of its course the furrow proceeds rapidly downward, constricting the surface of the egg, but its speed is decreased as it reaches the more rigid yolk in the vegetative hemisphere of the egg (fig. 23). The adjacent sides of the two furrows flatten against each other (text fig. D) to form a double cell-membrane, which extends from the surface of the egg inward to the remnant of the spindle between the two nuclei. Here and there spaces appear between the two membranes as if a drop of fluid separated them. Elsewhere the membranes are distinguished with difficulty.

The surface furrow gradually pushes through the remnant of the spindle from above downward and also extends across the bottom of the egg, but at the time that the chromosomes are formed on the spindle for the prophase of the second cleavage division the first furrow has not yet passed completely

FIGS. B-G. CLEAVAGE

Fig. B. Anaphase of first division in egg, *Triturus torosus*. $\times 66$.

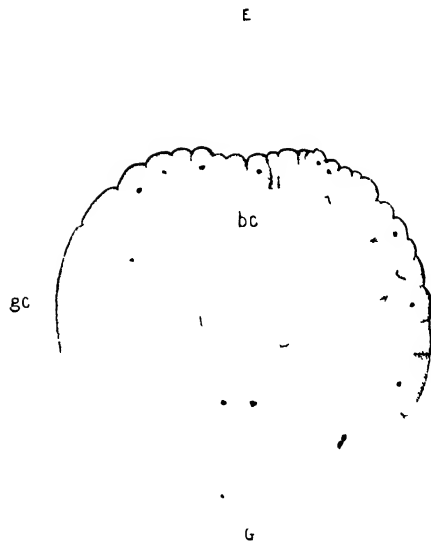
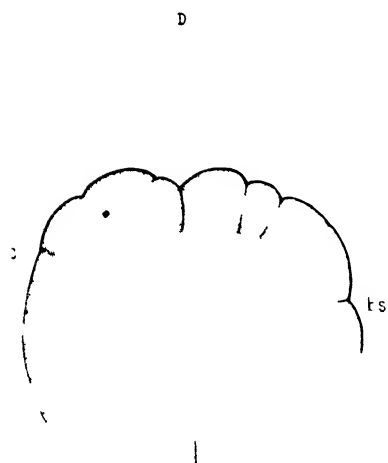
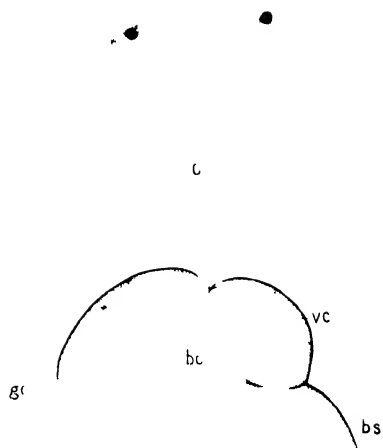
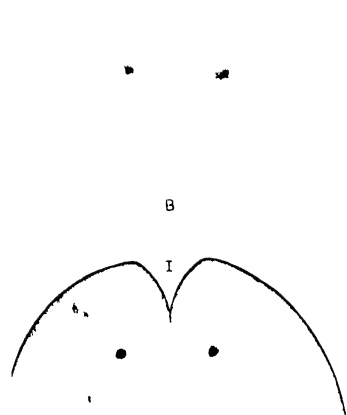
Fig. C. Telophase, showing pigment-free line extending across spindle to indentation in surface. $\times 66$.

Fig. D. Cleavage, showing first furrow (I) almost down to spindle. $\times 27.5$.

Fig. E. Eight-cell stage, sagittal section through gray crescent (gc.). $\times 27.5$. bc., blastocoele; bs., black shield; vc., vacuoles.

Fig. F. Thirty-two-cell stage, sagittal section through gray crescent (gc.). bs., black shield. $\times 27.5$.

Fig. G. Mid-blastula, sagittal section through gray crescent (gc.), showing single-layered roof and dividing floor of blastocoele (bc.). $\times 27.5$



Figs B-G Cleavage

through the yolk mass of the lower hemisphere, nor is it complete even in the telophase of the second division. The parts of the first furrow from above and below finally meet near the center of the egg after the nuclei attain the resting stage following second division.

During the prophase of the second division a space occurs internally between the two cell membranes and between the nuclei, which instigates the beginning of the second division. This space remains as a cavity which during later cleavages increases in size and becomes the important blastocoele (*bc.*, text fig. E) or segmentation cavity roofed over by cells.

Superficially the second cleavage furrow (II) appears at right angles to the first, and the resulting blastomeres thus outlined (fig. 24), if observed from the animal pole, are unlike those in *Amphioxus* (Cerfontaine, 1906; Conklin, 1932) in that they are essentially equal in size. The second plane passes downward and at the same time inward from the surface toward the center. After its completion the cytoplasm at the vegetative pole is carried well into the egg, as was shown by Schechtman (1934) in what he described as a type of polar ingression.

In third cleavage the spindles form vertically in the greater cytoplasmic mass, and consequently the third plane passes horizontally (text fig. E) and at right angles to planes 1 and 2, cutting off a quartet of smaller and more plastic blastomeres above from a quartet of larger and more rigid, yolk-laden blastomeres below. In *Triturus torosus* the anterior (ventral) micromeres do not bear a specific size relation to the posterior (dorsal) micromeres. They may be smaller than the posterior (dorsal) cells (text fig. E), or they may be larger (fig. 25). Between the larger micromeres and the opposite macromere there is often a so-called fifth cleavage furrow.

A section cutting the gray crescent sagittally during third cleavage shows the enlarging segmentation cavity or blastocoele (*bc.*, text fig. E) high in vertical axis, with its floor resting centrally on the more rigid yolk and its sides and thin roof formed of the more mobile cytoplasm. This mobile cytoplasm extends farther down than does the superficial layer of pigment in the black shield (*bs.*), and rises obliquely toward the side of the gray crescent (*gc.*). The vacuoles (*vc.*) which we observed to be formed in the zygote near the periphery of the animal area still persist as a marked feature at this stage.

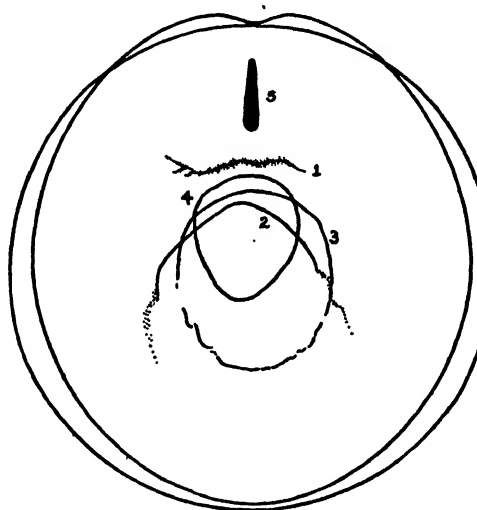
Figure 26 represents a typical fourth cleavage in which the eight smaller cells form a lozenge-shaped cap over the eight larger more yolk-laden cells, and figure 27 shows a side view of the beginning 32-cell stage. We have not followed surface cleavage beyond this stage.

A section through the 32-cell stage (text fig. F) gives essentially the same relations as those found in the 8-cell stage. The rigid yolk floor slopes upward toward the gray crescent (*gc.*), and the superficial pigment and more fluid cytoplasm extend farther down on the side of the black shield (*bs.*). The blastocoele, however, has extended in a horizontal direction and rests on the continuous yolk floor; the roof of the blastocoele is now formed of a layer of cells, some of which are small.

The cleavage pattern is fairly well established in text figure G and shows several advances over the conditions found in the 32-cell stage. The roof is now composed of many smaller cells, which, however, still form a single layer, and the yolk-laden cytoplasm of the floor is proliferating cells internally as well as completing its segmentation externally. Later development completes the pattern of the blastula by a further division of the cells of the roof so that at the end of blastulation these form a more or less complete double-layered roof (see fig. 28). The cells of the floor also divide repeatedly, and, where the animal and vegetative cells meet, the blastocoele dips down, so that the floor becomes convex in shape (fig. 28), as does the floor of the blastocoele in the *Anura*.

THE GASTRULA

The earliest external evidence of gastrulation in *Triturus torosus* is a slight depression near the margin of the ventral surface of the blastula (1, text fig. H). This depression occurs on the gray crescent side of the egg and pro-



H

Fig. H. Position of the blastopore in *Triturus torosus*, seen from below (ventral side) at different stages (1-5) in its development.

duces an elongate darkened area which becomes the dorsal part of the blastoporal groove. In vertical section in a plane which bisects this depression, that is, along the median sagittal plane of the embryo, the region is visible as a collection of pigment granules (*pg.*, fig. 28). These granules are situated in the converging tips of elongate club- or wedge-shaped cells, which have the enlarged ends stretching inward and the pointed ends converging toward the blastopore. The same section shows that above the beginning invagination on the side of the early dorsal lip the floor of the blastocoele is carried up slightly toward the roof, and that the region where the roof joins the floor of the blas-

tocoele is thinner at the end nearest the forming dorsal lip and thickest at the end farthest from the future dorsal region.

Superficially, as invagination progresses the blastopore becomes crescentic in outline (2, text fig. H), marking out the beginning archenteron, with the dorsal lip (*dl.*, fig. 28) above providing material which will later form the roof of the archenteron. Below the invagination (fig. 28) the yolk cells appear less active. In a later stage (fig. 29) the mass of material bounding the forming archenteron may be divided into three groups of cells: first, cells which compose the dorsal lip of the blastopore (*dl.*), some of which are in active division and appear to be piling up cells through the process of epiboly; second, active club-shaped cells (*cl.*) with their wide ends inward as if they were pulling inward against the looser cells of the blastocoele floor; and, third, the more static yolk-laden cells (*yk.*) which will invaginate to form the floor of the archenteron.

As the archenteron widens out (*ae.*, fig. 30), club-shaped cells no longer appear at its terminus, but cells somewhat like them are still to be found on the now deepening floor. The looser cells above the terminus of the archenteron become more and more dispersed and many of them at this time are in contact with the roof of the blastocoele on the blastoporal side. The primary roof of the archenteron extends inward by involution as its cells multiply slowly, and the surface area continues to add cells by rapid division in the upper part of the dorsal lip and to pass downward. Moreover, above the group of rapidly dividing cells the roof of the blastocoele, which in early gastrula stage was composed of a double layer of cells, now spreads out as a single layer, first in the area of the animal pole (fig. 29), and later far down into the dorsal lip (fig. 31). This spreading out of the roof cells doubles the surface and contributes largely to the piling up of cells in the dorsal lip.

By the time the blastopore has reached the shape of a third of a circle externally (3, text fig. H), the archenteron extends through the yolk mass, reducing the length of the blastocoele (fig. 31) at the same time that the height of the blastocoele is increased. This double effect is brought about largely by the pushing forward of the floor of the blastocoele in such a way that it doubles upon itself. As this process of doubling continues, the archenteron also greatly increases in height (*ae.*, fig. 32); this changes the center of gravity in the yolk, and the egg then undergoes secondary rotation (4 and 5, text fig. H), with the blastopore taking a horizontal position and the blastocoele being largely obliterated.

The shape of the blastopore and its ventral extent are not, however, absolute criteria for judging the sequence of stages in development. In a series of sections picked with blastopore formation as a criterion of development, some gastrulae in which the blastopores were more nearly circular showed less well advanced invagination of the yolk. In general, however, as involution of the dorsal lip progresses, the blastopore extends farther around to the side and is finally complete also ventrally, marking off the lateral and ventral lips (4, text fig. H; *vl.*, fig. 31), respectively. As the blastoporal lips approach one

another the yolk protruding becomes less extensive until only a small plug remains visible from the outside (*yp.*, fig. 32). Finally, this is drawn within the gastrula, leaving a slit-shaped blastopore (see 5, text fig. H, and *bp.*, fig. 42). We may regard the stage in which the egg has rotated so that the yolk plug is essentially in a horizontal position (fig. 32) as a completed gastrula.

THE PRIMITIVE ROOF OF THE ARCHENTERON AND ITS DERIVATIVES

In early stages of its development the roof of the archenteron immediately anterior to the dorsal lip of the blastopore, if viewed in sagittal section, is seen to form a layer several cells in thickness (fig. 29). Soon this material thins out somewhat (fig. 30), but it still forms with the overlying ectoderm a more or less common mass. Next, the roof in this view (fig. 31) appears as a club-shaped thicker posterior mass and an anterior part which is reduced to only a few cells in thickness and is separated from the single layer of overlying ectoderm. If seen in transverse section at this stage (figs. 34 and 38), it is observed that the roof proper consists of a median flattened part which is flanked right and left by somewhat thicker and more irregular parts. The flattened median dorsal section will give rise to the notochord (*ch.*), and the dorsolateral segments of the roof have in them two component parts, one of which (*ms.*, fig. 35) will give rise to musculature, and the other, the inner layer (*en.*), will form the permanent lining of the digestive tract. At this stage (fig. 31) the roof is also adding to itself anteriorly loose cells which will later continue forward from the prechordal plate (*ch.*¹, fig. 32) to terminate the anterior wall of the archenteron as a single layer of entodermal cell (*en.*).

THE NOTOCHORD

The notochord, as we have seen, is derived from the strip of material lying in the middorsal line of the roof of the archenteron (*ch.*, fig. 32). If studied in transverse sections, the cells composing the notochordal part of the roof are first observed to flatten out into a narrow plate only a few cells in width (*ch.*, figs. 34 and 35), which gradually extends forward. As development continues, the plate bends slightly downward (figs. 36 and 37) and then arches upward (fig. 39), so that from the archenteric cavity this notochordal plate would appear as a groove between right and left entodermal walls of the archenteron. The pillars of entoderm (*en.*, fig. 40) then approach each other, and the notochord as a distinct entity is lifted off the roof (see *ch.*, figs. 44 and 47). The yolk-laden entodermal cells right and left, which early formed the sides and part of the roof, join in the middorsal line to take the place occupied by the notochordal plate.

THE SOMITES AND THE LATERAL PLATE MESODERM

The mesoderm which gives rise to the somites may also form a portion of the roof of the archenteron in its early stages (*chm.*, fig. 40), but the greater part of it is separated from the archenteric cavity by the layer of entoderm (yolk cells). After the entoderm has bridged over the roof, upon the lifting up of the notochord all the dorsal mesoderm is separated from the archenteron (fig. 41) and gives rise to the somites.

When the invaginating yolk reaches approximately the center of the roof of the blastocoele, the mesoderm appears as a layer distinct from the entoderm and extends anteriorly about half the total distance from the dorsolateral part of the blastopore to the tip of the invagination. With the further development of the gastrula the right and left layers of mesoderm grow anteriorly and ventrally (fig. 34), but never meet in the middorsal line where the notochordal part of the roof of the archenteron is in contact with the ectoderm (fig. 39). In the late yolk-plug stage the dorsolateral mesoderm extends anteriorly almost to the middle of the gastrula; at this stage ventrolateral mesoderm also is evident in the posterior part, as is shown in frontal and transverse sections (*ms.*, figs. 33 and 34, respectively). The ventrolateral mesoderm is early separated into outer and inner layers, between which is later formed the body cavity.

When the blastopore has closed to a vertical slit (fig. 42; 5, text fig. H), the dorsolateral mesoderm extends on each side more than three-quarters of the way forward from the blastopore to the front of the embryo; and the mesoderm at the midventral line extends about one-third of that distance. When the notochord is almost separated from the archenteron, the lateral mesodermal bands nearly meet anteriorly, and ventrally the mesoderm extends more than half the distance to the anterior end. After the notochord is completed and the ventral mesoderm has extended anteriorly to the floor of the archenteron, where the stomodeum will form, the lateral bands meet in front of the ventral portion of the pharyngeal region, behind the ventral end of the future forebrain, and the dorsolateral mesoderm (fig. 44; *so.*, fig. 47) begins to form somites.

THE NEURULA

In the late gastrula the surface ectoderm immediately anterior to the dorsal lip of the blastopore shares in the flattening of the notochordal plate (fig. 34). Sections show that this flattened area is composed of a single layer of cells over the region of the archenteric roof. The roof, as we have seen, is formed of cells of the notochord (*ch.*, fig. 35), mesoderm (*ms.*), and entoderm (*en.*) at this stage, the notochord and mesoderm being in close contact with the ectoderm (*ec.*, fig. 36). Beginning near the posterior end and gradually extending anteriorly, the center of the flattened area of ectoderm sinks down and becomes thinner (figs. 36 and 37), later forming a median longitudinal groove above the notochord.

Externally the earliest indication of neural folds or ridges are pigmented streaks extending anteriorly on both sides of the blastopore about midway

between the longitudinal neural groove and the equator. In cross sections the ectoderm where the folds later appear is slightly thicker than elsewhere (*nr.*, figs. 36 and 38), the thickening being more pronounced anteriorly than posteriorly. It is important to note that underlying the thickening ectoderm are groups of mesodermal cells (*ms.*, fig. 38) which appear to bear a causal relation to the thickening. Gradually the ectoderm of the entire region between the ridges, except that in the longitudinal groove, becomes thicker than elsewhere. This early thickening is due to an elongation of the ectodermal cells above the mesoderm rather than to an increase in the number of cell layers. Later its wall becomes many cells in thickness. Thus there is formed on the dorsal surface of the embryo a medullary plate of thickened ectoderm (fig. 43) which widens out anteriorly and extends down over the end to a point at or below the equator, where right and left ridges join as a transverse ridge (fig. 45). In surface view the medullary plate (*mp.*) is first marked off by these ridges as a horseshoe-shaped area which later becomes spatulate.

As the ridges thicken they become the neural folds (*nf.*, fig. 43); the folds grow upward, and pull the thinner ectoderm (*ec.*) along with them. The thinner ectoderm of the outer edges of the folds becomes continuous over the top of the embryo (*ec.*, figs. 44 and 47) and the sides of the neural plate close over the longitudinal groove, which then forms a part of the floor of the neural canal; the other medullary plate cells form the sides and roof. The wider anterior part of the plate, which gives rise to the brain, closes latest over a larger cavity, the future ventricles of the brain. From the posterior part of the plate the spinal cord arises.

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EXPLANATION OF PLATES

PLATE 53

Fig. 1. Ovarian egg of *Triturus torosus* from section of ovary cutting through germinal vesicle (*gv.*). $\times 18$.

Fig. 2. Detail of surface of ovarian egg at vegetative pole, showing coverings and membranes. *cm.*, cyst membrane; *fc.*, follicular cells; *oe.*, ovarian epithelium; *sp.*, zona pellucida; *sr.*, zona radiata. $\times 302$.

Fig. 3. Section through germinal vesicle (*gv.*), showing nucleoli and scattered granular threads. $\times 75$.

Fig. 4. Coelomic egg. The germinal vesicle has ruptured but has not completely disappeared. The chromatin is massed in two irregular clumps (*cr.*). One large nucleolus (*ns.*) is present, surrounded by a faint halo of hyaline protoplasm. The spindle has not yet formed. $\times 318$.

Fig. 5. Coelomic egg. There is still some yolk-free space which indicates the remnant of the germinal vesicle. The first spindle (*sd.*¹) is forming and the chromatin in characteristic tetrad shapes is becoming aligned on it. $\times 762.5$.

Fig. 6. Coelomic egg. Chromosomes on first maturation spindle (*sd.*¹). Chorion removed. At left, a large nucleolus (*ns.*) lies near the surface in a clear area. One or more of these may be present until after first division occurs. Stage found in many coelomic eggs and in eggs from upper oviduct. $\times 710$.

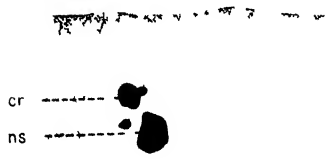
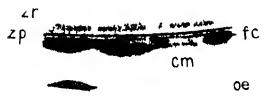
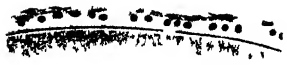
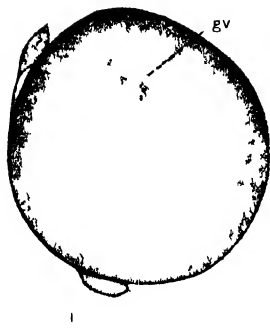


PLATE 54

Fig. 7. External view of egg of *Triturus torosus* from lower part of oviduct, studied in normal salt solution to show its surrounding jelly layers. *ch.*, chorion; *gr.*, germinal area; *j.¹⁻⁴*, first to fourth jelly layers.

Fig. 8. Detail of jelly from an egg fixed and sectioned in place in lower oviduct. *j.¹⁻⁴*, first to fourth jelly layers. $\times 68$.



Fig. 9. First polar body (*pb.*¹) being extruded from coelomic egg of *Triturus torosus*. Egg studied in saline solution for two hours after removal from coelome. When first observed, only an indistinct mass of pigment visible within the germinal area. When fixed, distinct ring of pigment, collected thickly around spindle, seen in germinal area. Chorion (*ch.*) pulled up by sectioning so that wide piece shown represents flat strip rather than cross section. $\times 710$.

Fig. 10. Second maturation spindle (*sd.*²) in coelomic egg. Chromosomes, with beaded appearance, lie in paired V's, and first polar body (*pb.*¹) occupies depression on surface under the chorion. Of 93 coelomic eggs examined, 44 per cent were in approximately this stage. Second maturation spindle remains in this condition until fertilization or disintegration. $\times 710$.

Fig. 11. Second polar division in an egg fixed shortly after laying. Several spermatozoa present deeper in cytoplasm. *ch.*, chorion; *pb.*², second polar body. $\times 762$.

Fig. 12. Second polar division in another egg fixed shortly after laying. Chorion lost. Material around second polar body (*pb.*²) is coagulated fluid in space between chorion and egg. $\times 762$.

Fig. 13. Telophase of second polar division. Note cross-striated (*cs.*) egg surface above chromosomes. Second polar body (*pb.*²) under chorion. $\times 762$.

Fig. 14. Egg pronucleus forming under striated surface (*cs.*) after second polar division. $\times 762$.

Fig. 15. Early egg pronucleus (*pn.*). Surface striations of egg have disappeared. Pronucleus moving inward. $\times 762$.



PLATE 56

Fig. 16. Sperm entering egg of *Triturus torosus*. Head completely inside. Middle piece, or neck, and tail (*tl.*) outside egg. Chorion removed during preparation of egg for sectioning. Vacuolation (*vu.*) of surface disappears soon after sperm enters. Drawing made from two adjacent sections; everything but tail drawn from one section. $\times 762$.

Fig. 17. Entire sperm within egg, forty minutes after laying. Nucleus of egg same stage as that shown in figure 13, with second polar body formed. Sperm drawn from several consecutive sections; adjacent portions matched by means of camera lucida. $\times 302$.

Fig. 18. Early transformation of sperm head. Dark bend in center indicates where drawing is pieced together from two sections. Sperm head entered from above, but aster (*ar.*) now precedes; smaller end nearer place of entrance. $\times 762$.

Fig. 19. Sperm pronucleus (σ) and penetration path shown by arrow. $\times 302$.

Fig. 20. Two pronuclei nearly together (sperm pronucleus smaller). $\times 318$.

Fig. 21. Irregular sperm path and two pronuclei in contact (sperm pronucleus smaller).



PLATE 57

Fig. 22. Beginning of two-cell stage in egg of *Triturus torsus*, showing hummocks (*hm.*). Gray crescent to left. Dorsal view. $\times 22$.

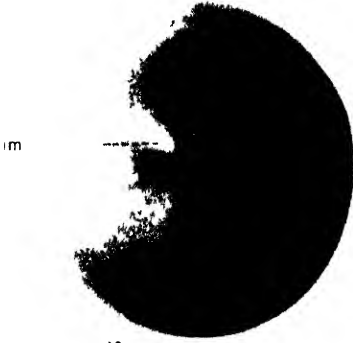
Fig. 23. Two-cell stage, dorsal view. Gray crescent side to left. $\times 22$.

Fig. 24. Beginning of second cleavage plane (II) superficially at right angles to the first (I); dorsal view. $\times 22$.

Fig. 25. Eight-cell stage, with the gray crescent (*gc.*) turned to left, showing micromeres above, macromeres below, and fifth furrow between larger micromere and macromere. *bs.*, black shield. Lateral view. $\times 22$.

Fig. 26. Sixteen-cell stage, dorsal view. $\times 22$.

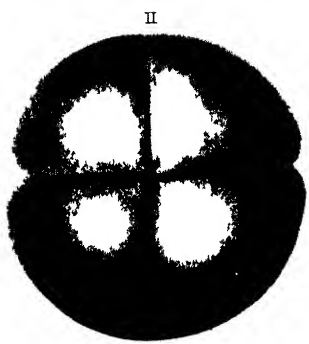
Fig. 27. Beginning thirty-two cell stage with black shield (*bs.*) to right and gray crescent (*gc.*) to left. Lateral view. $\times 22$.



22



23



24



25



26



27

PLATE 58

Gastrulae

Fig. 28. Sagittal section of young gastrula of *Triturus torosus*, showing pigmented (*pg.*) ends of club-shaped cells (see *cl.*, fig. 29) and slight external groove. Note also double-layered roof of blastocoele (*bc.*). *dl.*, dorsal lip of blastopore. $\times 25$.

Fig. 29. Sagittal section of stage in which invagination extends about one-fifth the distance to roof of blastocoele. *cl.*, club-shaped cells at inner end of invagination; *dl.*, dorsal lip of blastopore; *yk.*, yolk-laden cells. $\times 25$.

Fig. 30. Sagittal section of stage in which invagination extends about half-way to roof of blastocoele, which is again single layered. No elongate cells at end of archenteron (*ac.*). Cells along floor somewhat elongate. *dl.*, dorsal lip of blastopore. $\times 25$.

Fig. 31. Archenteron (*ac.*) widening. Yolk cells of floor bending up toward roof of blastocoele (*bc.*). *dl.* and *vl.*, dorsal and ventral lips of blastopore, respectively. $\times 25$.

Fig. 32. Late yolk-plug stage. Blastocoele nearly obliterated. *ac.*, archenteron; *ch.* and *ch.*¹, chordal and prechordal plates, respectively; *ec.*, ectoderm; *en.*, entoderm forming anterior margin of archenteric wall; *yp.*, yolk plug. $\times 25$.

Fig. 33. Frontal section through blastopore. Very late gastrula, with slight flattening anterior to blastopore. Shows extent of lateral mesoderm (*ms.*) at this stage. *ac.*, archenteron; *bc.*, blastocoele; *ll.*, lateral lips of blastopore. $\times 25$.

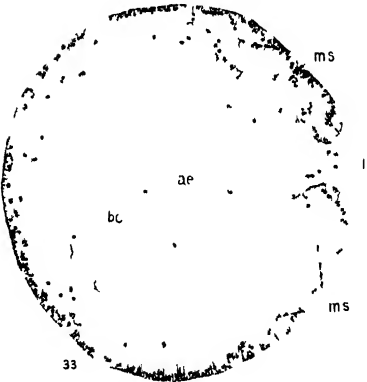
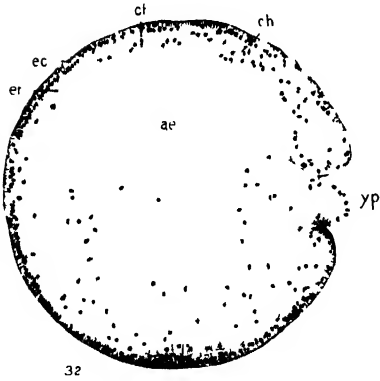
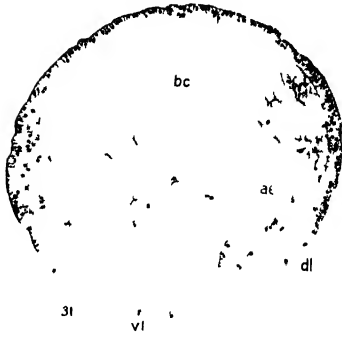
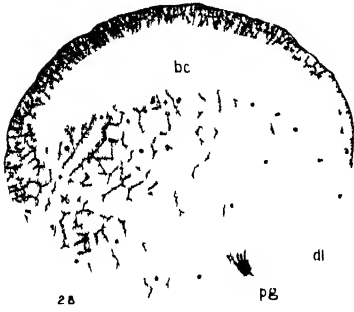


PLATE 59

Neurulae

Fig. 34. Cross section of late gastrula at time when slight flattening appears anterior to blastopore. Section shown is 61st from posterior end. *ac.*, archenteron; *bc.*, remnant of blastocoel; *ch.*, notochord; *en.*, entoderm; *ms.*, ventral extent of mesoderm. $\times 25$.

Fig. 35. Late gastrula, 25th cross section from posterior end. Shows flattening of dorsal region anterior to blastopore, and thinning of cells where neural groove will form. *ch.*, notochordal plate; *en.*, entoderm; *ms.*, mesoderm. $\times 39$.

Fig. 36. Neural groove stage, 55th section from posterior end. Mesoderm (*ms.*) complete ventrally; ectoderm (*ec.*) thicker in dorsal half of embryo, especially where neural ridges or folds (*nr.*) will form. *ch.*, notochord. $\times 25$.

Fig. 37. Neural groove stage, 32d cross section from posterior end. Mesoderm (*ms.*) extends to ventral side and forward to 66th section. A few mesoderm cells in dorsolateral region seen as far forward as 126th section. *ch.*, notochord. $\times 39$.

Fig. 38. Neural groove stage, 120th section from posterior end. Scattered mesoderm cells (*ms.*) seen in dorsolateral region, under thickening ectoderm of early neural ridges (*nr.*). *ch.*, notochord. $\times 25$.

Fig. 39. Cross section of early neurula, at stage when lines of pigment only mark regions where neural folds form, 83d section from posterior end. *ch.*, chordal plate. $\times 39$.

Fig. 40. Fortieth cross section from posterior end, showing chordamesoderm plate (*chm.*) as roof of archenteron. *en.*, entoderm; *ms.*, mesoderm. $\times 39$.

Fig. 41. Sixty seventh cross section from posterior end. Neural folds (*nf.*) are beginning to show; they are more distinct as ridges posteriorly. Notochord cells (*ch.*) being closed out of archenteron (*ac.*). $\times 39$.

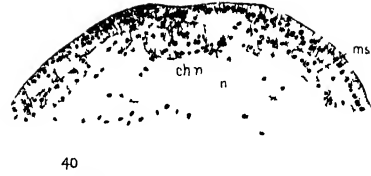
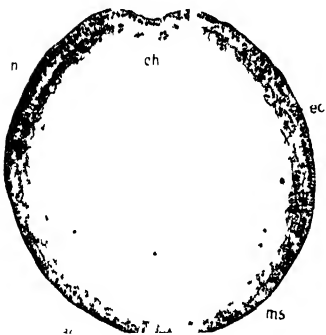
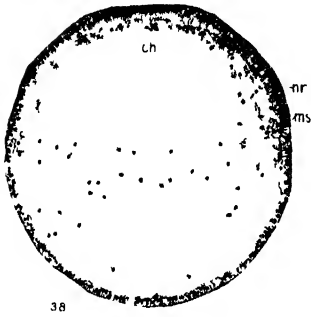
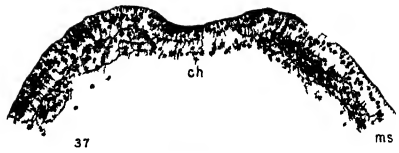
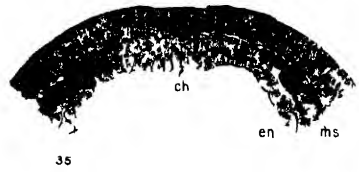
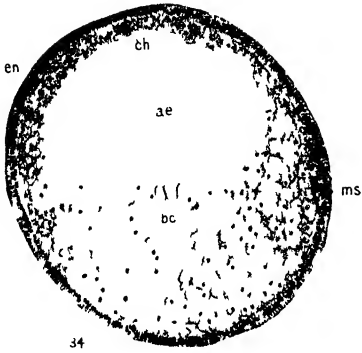


PLATE 60

Neurulae

Fig. 42. Median sagittal section, early neural fold stage. *ac.*, archenteron; *bp.*, blastopore; *ms.*, mesoderm; *tf.*, transverse neural fold. $\times 25$.

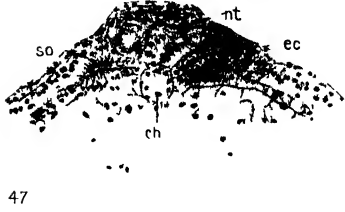
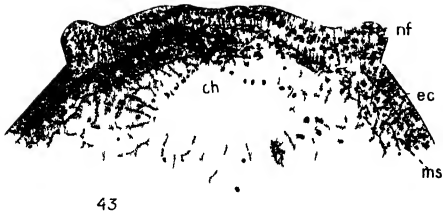
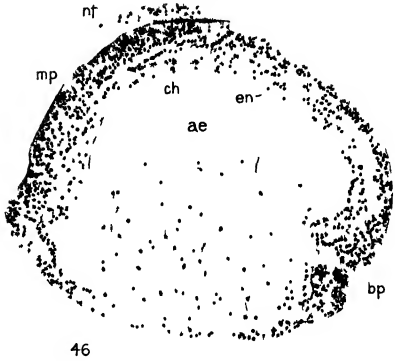
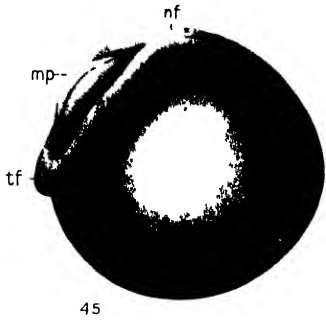
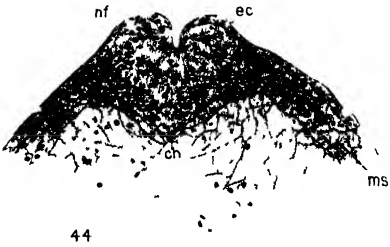
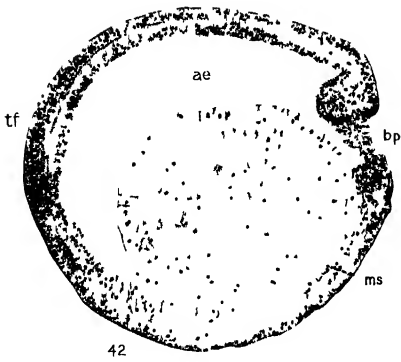
Fig. 43. Mid-neurula stage, 76th cross section from posterior end, showing neural folds (*nf.*) rising; two layers of mesoderm (*ms.*); *ec.*, ectoderm; notochord (*ch.*) closing out of archenteron. $\times 39$.

Fig. 44. Medium late neurula, 81st cross section from posterior end. Neural folds (*nf.*) nearly closed. Thinner ectoderm (*ec.*) extends over tops of neural folds. Notochord (*ch.*) completely separated from archenteron. *ms.*, mesoderm. $\times 39$.

Fig. 45. Whole mount, medium late neurula, lateral view. *mp.*, medullary plate; *nf.*, lateral neural fold; *tf.*, transverse neural fold. $\times 17$.

Fig. 46. Median sagittal section, medium late neurula. See figure 44 for cross section, same stage. *ac.*, archenteron; *bp.*, blastopore; *ch.*, notochord; *en.*, entoderm; *mp.*, medullary plate; *nf.*, lateral neural fold. $\times 25$.

Fig. 47. Late neurula, 83d cross section from posterior end. Neural tube (*nt.*) completely closed. *ch.*, notochord; *ec.*, ectoderm; *so.*, somite. $\times 39$.



STUDIES OF SOME AMOEBAE
FROM A TERMITE OF
THE GENUS CUBITERMES

BY

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INTRODUCTION

SIX SPECIES of amoebae have been reported from termites, all of which, together with those here described, have been found in the family Termitidae. Kirby (1927, 1932) described five of them from two species of *Mirotermes*, and one from five species of *Amitermes*.

Material for the present study was collected by Professor Harold Kirby, Jr., from a colony of *Cubitermes* n. sp.¹ near Nairobi, Kenya Colony. Smears were prepared in the field and fixed in Schaudinn's fluid. They were later stained in Delafield's or in Heidenhain's iron-haematoxylin. Living material was not available for systematic study, but I am indebted to Professor Kirby for field notes on living forms.

Study of the stained material revealed four new species of entozoic amoebae together with fairly numerous trichomonad flagellates. Also there were noted at least one species of smaller flagellates, numerous filamentous bacteria and spirochaetes, and matter which appeared to be earthen particles, plant spores, and fragments of plant tissue. A count made of twenty-four smears gave 3140 amoebae and 7297 trichomonads. Thus, the average number of amoebae per slide was 131, though smears differed considerably in the number of amoebae present. The maximum number on one smear was 436.

Thanks are due to Professor Kirby, under whose direction the work was done, not only for making the material available but also for advice and very helpful criticism, and to Mr. Carl M. Stover for advice in the technique of drawing and preparing the plates.

DESCRIPTION OF SPECIES

Endamoeba pellucida n. sp.

(Pl. 61, figs. 1-10)

Type host.—*Cubitermes* n. sp. T-2039. Kamiti River, near Nairobi, Kenya Colony (syn-type slides TP-2001:13, 14, 17, 24).

Diagnosis.— 13.5×14.5 – $29.5 \times 32.5\mu$, averaging $21 \times 22.5\mu$; spherical, ovoidal, or broadly ellipsoidal; cytoplasm finely granular and vacuolated, no pseudopodia, ingested particles few; nucleus ellipsoidal, 5.5×7 – $9 \times 11.5\mu$, averaging $7 \times 9\mu$; heavy nuclear membrane with usually two peripheral granules flattened against it; central mass of chromatic granules with karyosomal dot.

This species, comprising approximately 57 per cent of the amoebae counted, is not only the most numerous but also the most uniform in size, shape, and general appearance. Individuals less than 20μ or more than 25μ in length are

¹ The termite was determined as a new species of *Cubitermes* by Professor A. E. Emerson.

infrequent. There is generally no differentiation of the cytoplasm, ectoplasm being rarely distinguishable; and pseudopodia appear to be entirely absent, at least in fixed material. The body, bounded by a distinct membrane, appears at all times sharply defined, never being obscured by adherent debris as in larger species in the same smears. The cytoplasm is alveolar and finely granular. Numerous individuals contain ingested trichomonads in various stages of digestion (pl. 61, fig. 3), usually not more than one to a specimen but occasionally as many as three. In addition to these inclusions, filaments, minute rods, and small, deeply stained bodies—all of uncertain nature (possibly bacteria)—are frequently found. But ingested matter is never present in great abundance, so that the vacuolated cytoplasm exhibits nearly always a clear and uniform appearance.

From field observations made on living material, Professor Kirby notes the occurrence and activity of "smaller, transparent amoebae," which, considering their appearance, relative size, and abundance, could hardly have been other than this species. Locomotion was observed to be by fountain streaming, the protoplasm flowing in a broad tube down the center toward the anterior end and returning at the periphery. Pseudopodia were not formed, and very little clear ectoplasm was observed.

The nucleus is ellipsoidal in shape and always eccentric in position (pl. 61, fig. 1). The nuclear membrane is heavy and distinct, and, except for one or two chromatic granules flattened against it, is of even contour. The chromatic granules are typical of this nucleus. There are usually two of them, on opposite sides of the nucleus (pl. 61, fig. 2). They stain deeply and evenly in iron-haematoxylin; although not so conspicuous in Delafield's haematoxylin, they can still be clearly seen. Apparently there are never more than two of these bodies; a few nuclei do not show them, and some show only one (pl. 61, fig. 5). The inner zone of the nucleus is occupied by a granular chromatin mass, within which there can usually be seen a central karyosomelike granule (pl. 61, fig. 3). The other granules approach this central one in size, but they can be distinguished from it by their less regular outlines and lighter staining. The central granule cannot be distinguished in Delafield's haematoxylin, nor always in Heidenhain's. It is possible that it might be overlooked because of an overlying position of other granules or lack of differentiation in the staining. It will be noted that it does not occur in such nuclei as are shown in plate 61, figures 9 and 10, which are presumably in early stages of karyokinesis. The granular mass is usually compact and central, but occasionally it is so diffuse as to occupy nearly the entire nucleus (pl. 61, fig. 4). Between the central mass and the nuclear membrane there is a clear zone, across which filaments can frequently be traced; some pass to the peripheral granules, and others terminate at the membrane itself.

Unfortunately, the course of mitosis could not be traced in this species. Nuclei like that shown in plate 61, figure 10, are of fairly common occurrence, but inasmuch as stages transitional between them and other forms are lacking, their significance is obscure. The spireme nucleus of plate 61, figure 9, was the only one of its type observed. It suggests the formation of chromo-

somes; however, in three probable anaphases, not sufficiently clear for accurate observation, definite chromosomes were not observed. Apparently there were merely masses of chromatin granules at each pole.

No cysts have been found.

Endamoeba granosa n. sp.

(Pl. 62, figs. 18–26)

Type host.—*Cubitermes* n. sp. T-2039. Kamiti River, near Nairobi, Kenya Colony (syntype slides TP-2001:13, 14, 17, 20).

Diagnosis.— $16 \times 16\text{--}24 \times 97\mu$, averaging $32.5 \times 43.5\mu$; spherical, ellipsoidal, or oblong; cytoplasm coarsely granular, ingested particles large and numerous; nucleus usually ellipsoidal, $5.5 \times 5.5\text{--}14.5 \times 20\mu$, averaging $9 \times 11.5\mu$; one or more large granules or plaques against the nuclear membrane; inner zone of nucleus occupied by chromatic granules, which may be aggregated or arranged in lines; karyosome with halo usually apparent.

This species, the second most abundant in the intestine of its host, is the most diverse in size and appearance. It comprises approximately 33 per cent of the amoebae observed. The smallest specimens are usually spherical or broadly ellipsoidal; the larger forms are irregular in shape but tend to be ellipsoidal or oblong. One end of the animal is frequently coated with adherent debris, which is so thick at times as to make the body outline indistinguishable. The mode of locomotion of this species, as recorded in field notes, is essentially like that observed in *Endamoeba pellucida*, that is, by fountain streaming.

The cytoplasm presents a coarsely granular appearance and is usually crowded with ingested particles, frequently of large size (pl. 62, fig. 18). They appear to be, for the most part, plant spores and fragments of plant tissue. Ectoplasm is apparent in one or more broad lobes (pl. 62, figs. 18 and 26), and in certain of the smaller individuals as a broad zone almost surrounding the endoplasm (pl. 62, fig. 25).

Except in the smallest specimens, where it is nearly spherical, the nucleus is ellipsoidal. The nuclear membrane is relatively thin but distinct. Adhering to its inner surface are one or more plaques or flattened granules that stain in different degrees of intensity and frequently not homogeneously (pl. 62, fig. 21). A large part of the nucleus is occupied by a central mass of chromatic granules, which vary in size in different nuclei (pl. 62, figs. 18–20) but tend to be of the same size in a given nucleus. They are sometimes in a compact mass (pl. 62, fig. 18), sometimes diffuse (pl. 62, fig. 21), sometimes arranged in closely packed threads, so dense as to make the structure difficult to resolve (pl. 62, fig. 25). Between such arrangements of the granules, transitional forms are fairly common (pl. 62, figs. 19 and 22). Nuclei of the smaller forms (pl. 62, fig. 24) usually present a central mass so deeply stained that their detailed structure cannot be distinguished, but in a few examples the stain has been sufficiently extracted to show a granular structure similar to that of the larger nuclei. Within the granular mass—more especially in the larger and clearer nuclei—there is frequently to be seen a large, rounded, deeply stained granule, surrounded by a clear space and connected with the smaller granules by radial filaments (pl. 62, figs. 18–21). Between the chromatin mass

and the nuclear membrane, fibers and lines of minute granules are commonly seen, terminating both in the membrane and in the peripheral plaques.

A single, somewhat imperfect cyst was found (pl. 62, fig. 23). Unfortunately, the nuclei were so deeply stained as to make details of their structure impossible to resolve. From its size, its cytoplasm, and what could be seen of its nuclei (peripheral plaques, large, dense chromatin mass), it appears to belong to this species. Making allowance for the imperfect staining, one observes that the nuclear structure of the cyst presents a similar picture to that of the trophozoite shown in plate 62, figure 24.

Endamoeba lutea n. sp.

(Pl. 63, figs. 27-35)

Type host.—*Cubitermes* n. sp. T-2039 Kamiti River, near Nairobi, Kenya Colony (syn-type slides TP-2001:13, 14, 22, 24).

Diagnosis.— $25 \times 34-76 \times 151\mu$, averaging $48.5 \times 64\mu$; spherical, ellipsoidal, or irregular in shape; cytoplasm coarsely granular, ingested particles numerous; nucleus spherical, $5.5-17\mu$ in diameter, averaging 11μ ; chromatin mass a granular reticulum, which may fill nucleus but is usually partly surrounded by a clear space; two to nine deeply staining, rounded bodies scattered throughout the nucleus.

Amoebae of this species are of relatively infrequent occurrence, accounting for but 6 per cent of the total number. Even in stained material the body presents a yellowish brown appearance due to numerous ingested particles and to masses of adhering debris. Ectoplasm can usually be seen in one or more narrow lips on the body surface (pl. 63, figs. 27-28), or at times, especially in well-rounded forms, as a narrow rim expanded at one end into a broad zone (pl. 63, fig. 32). The cytoplasm is filled with ingested particles (pl. 63, fig. 32) and contains many coarse granules. Owing to its relatively small numbers, it is not certain that this species was observed in living material.

The nucleus is so frequently spherical that occasional nuclei of broadly ellipsoidal outlines can possibly be attributed to distortion. The nuclear membrane is distinct but not especially heavy and encloses a finely granular reticulum, which may fill the entire nucleus (pl. 63, fig. 29) but which is more often contracted, either to one side, remaining in broad contact with the membrane, or toward the center. Distributed on the reticulum are numerous small chromatin granules, which at times arrange themselves in lines or form a kind of loose network superimposed on the finer structure (pl. 63, figs. 34-35). The remaining, clear part of the nucleus is crossed by rather numerous filaments, lines of minute granules, and at times a network of fine particles. Always present in this nucleus are from two to nine (usually five to seven) deeply staining spheres or ellipsoids, apparently nucleoli, distributed throughout the nuclear space (pl. 63, fig. 29). Occasionally one or more of them can be seen flattened against the nuclear membrane, but that position is not characteristic. They vary in size from small granules to bodies of a diameter of 3.5μ . One of them is typically larger than the others and is likely to be irregular in outline and unevenly stained, appearing as a thick ring in optical section.

Endolimax suggrandis n. sp.

(Pl. 61, figs. 11-17)

Type host.—*Cubitermes* n. sp. T-2039. Kamiti River, near Nairobi, Kenya Colony (syntype slides TP-2001:1, 8, 12, 14).

Diagnosis.— 10×11 – $11 \times 48.5\mu$, averaging $12.5 \times 19\mu$; shape variable, tending to be elongated; smooth surface; cytoplasm finely granular and vacuolated, ingested particles few; nucleus spherical, 3.5 – 5.5μ in diameter, averaging 4.5μ ; nuclear membrane delicate and indistinct, incrustated with fine chromatin granules; large, roughly spherical endosome, differentiated into large chromatic particles and achromatic ground substance, surrounded by radial filaments.

This species is the smallest of the amoebae present in the intestine of its host and the least numerous, amounting to approximately 4 per cent of the total number. Its shape varies within wide limits; it is sometimes spherical, but is most commonly considerably elongated and irregular. Individuals longer than 25μ are rare, but one specimen was found with the rather surprising dimensions of $48.5 \times 11\mu$. The cytoplasm presents an appearance very similar to that of *Endamoeba pellucida* (to which it is obviously unrelated), being finely granular and vacuolated and comparatively free from ingested particles. A few forms were seen with fairly large inclusions (pl. 61, fig. 15) and with long filaments in the cytoplasm (pl. 61, fig. 17), but they were exceptions. Differentiation of the cytoplasm seems to be lacking except as exhibited by the presence of pseudopodia, which is rare.

The nucleus is spherical and almost constant in size, regardless apparently of the variation in body size. Of 55 nuclei measured, 43 were 4.5μ in diameter. The nucleus of the largest form ($48.5 \times 11\mu$) still measured only 4.5μ . The nuclear membrane is delicate, though the presence on its inner surface of numerous fine chromatin granules gives an illusion of thickness. Almost filling the nucleus is a large, roughly spherical endosome, which appears to consist of an achromatic ground substance in which are embedded chromatic granules (pl. 61, fig. 12). The chromatic particles of the endosome vary considerably in size and appear to be larger at its periphery. Although the center of the mass is usually occupied by an assortment of small granules, a single central one surrounded by a clear space (such as was sometimes observed by Kirby in *Endolimax termitis*) could not be seen in this species. Surrounding the endosome is a narrow clear zone crossed by radial filaments.

The present form resembles *Endolimax termitis* Kirby rather closely. Body and nucleus, respectively, are virtually of the same average size, and the general appearance is similar. After a comparison of the two forms in stained material, specific distinction seems justified on the basis of the following morphological differences: (1) The peripheral chromatin of *E. termitis* is in comparatively large granules, which, probably, because of their size, give the nuclear margin the appearance in optical section of a deeply staining ring. The peripheral chromatin of *E. suggrandis* is in much smaller granules, and the boundary of the nucleus is not conspicuous, being rather difficult to distinguish. (2) The endosome of *E. termitis* is small as compared with that of *E. suggrandis*, its diameter being a little more than half that of the nucleus,

whereas the endosome diameter in the latter species is a little more than three-fourths that of its nucleus. This difference can be seen on comparing figures illustrating the two forms and is readily apparent on inspecting the material. (3) The chromatin granules in the endosome of *E. termitis* are comparatively small and occupy a relatively small portion of that body; those of *E. suggrandis* are large, especially at the periphery, and occupy the greater part of the endosome. (4) The occasional central granule surrounded by a clear space, found in *E. termitis*, was not found in *E. suggrandis*.

DISCUSSION

Inasmuch as knowledge of the present species is necessarily limited because it is not possible to study the complete life histories, any attempt to do more than suggest possible phylogenetic relationships would be premature. Mitotic stages are generally lacking, and cysts, which are always of taxonomic importance, have been found only once.

With the exception of *Endolimax suggrandis*, reasons for the specificity of which have already been outlined, none of the present forms appear to be sufficiently similar to previously described species to raise doubts of specific distinction. Nevertheless, it would not be surprising to find similarities between these forms from *Cubitermes* and other species from hosts of the same family of termites. Such indications of relationship appear to be present, with respect not only to other amoebae of termites, but also to forms found in the cockroach.

Including those here reported, thirteen species of entozoic amoebae have been described from termites and cockroaches. They are the following: *Endamoeba blattae* (Bütschli, 1878) Leidy, 1879, from *Blatta orientalis*; *Endamoeba thomsoni* Lucas, 1927, and *Endolimax blattae* Lucas, 1927, from *Blatta orientalis* and *Periplaneta americana*; *Endamoeba disparata* Kirby, 1927, *Endamoeba majestas* Kirby, 1927, and *Endolimax termitis* Kirby, 1927, from *Mirotermes hispaniolae*; *Endamoeba simulans* Kirby, 1927, and *Endamoeba sabulosa* Kirby, 1927, from *Mirotermes panamaensis*; *Endamoeba beaumonti* Kirby, 1932, from *Amitermes beaumonti*, *A. coachellae*, *A. minimus*, *A. wheeleri*, and *A. medius*; and the four species here described. To facilitate comparison, a table is given listing the forms named above with their outstanding morphological characteristics. This table is based upon the original description of each species except for *Endamoeba blattae*, which is based upon a recent account of that form by Morris (1936).

The forms listed in the table tend to fall into three rather distinct size groups with respect to body and nuclear dimensions. These groups have been separated by double lines. Similarities, at least in the first and third groups, are not limited to size, but obtain also in nuclear structure.

Kirby (1927) directed attention to a morphological relationship between *Endamoeba disparata* and *E. sabulosa* based upon similarity in size and nuclear dimensions, and the possession by both species of a delicate nuclear membrane, of an irregular granular mass of chromatin which nearly fills the interior of the nucleus, and of several irregular, deeply staining nucleolar

bodies peripheral to the mass (just under the nuclear membrane in *E. disparata* but against the mass itself in *E. sabulosa*). In the same communication he noted the similarity of *E. disparata* in size and nuclear structure to an amoeba in the cockroach, apparently the form that was described by Lucas as *Entamoeba thomsoni* at about the same time. Later, in describing *Endamoeba beaumonti*, Kirby (1932) noted the resemblance of that amoeba to *Entamoeba thomsoni* and *Endamoeba disparata*, based on the same features that were common to the latter and *E. sabulosa*.

Endamoeba pellucida differs from this group mainly in the possession of an ellipsoidal nucleus and a thick nuclear membrane. It agrees with the other members of the group in size and in having a nucleus with peripheral granules and largely occupied by a granular chromatin mass. The central karyosomal granule, a conspicuous feature of *E. pellucida*, is noted in all other amoebae of this group except the first two. The peripheral granules are against the nuclear membrane, as in *E. pellucida*, in all other amoebae of the group except *E. sabulosa*. A further basis of possible relationship is afforded by the fact that in *E. disparata* about half the interphase nuclei were found to be fusiform, which suggests a connection with the ellipsoidal nucleus of *E. pellucida*.

Within the second group, morphological similarities are not so apparent. Probably the best-known amoeba there listed is the type species of the genus *Endamoeba*, *E. blattae*, which will serve as a convenient basis for comparison. This amoeba has been the subject of numerous investigations, the more recent of which are in substantial agreement with respect to the structure of the resting adult nucleus, though they differ in interpretations of the life cycle. The reader is referred to the summary given by Wenyon (1926) and to more detailed accounts by Mercier (1910), Kudo (1926), Thomson and Lucas (1926), and particularly Morris (1936), who has made a recent and thorough study of this form.

The nucleus of the adult *E. blattae* is described by Morris as sometimes spherical but more often slightly elongated, of diameter from 10 to 25 μ , and invested by a membrane 1 μ thick. Within the nuclear membrane is a broad zone, granular in living material, fibrous in fixed material; and within this fibrous zone is a central region which is densely reticular in fixed material and within which a dotlike karyosome is sometimes apparent. The outer, fibrous zone shows numerous chromatic granules in the fixed state and "large nucleolus-like bodies which give rise to the pseudo-chromosomes." Concerning the nature of these last-mentioned elements there seems not to be general agreement. Morris states that they are formed by coalescence of peripheral chromatin in postmitotic reorganization, and Wenyon also calls them chromatic granules, whereas others have regarded them as nucleoli.

Kirby (1927) compared *Endamoeba majestas* and *E. simulans* with *E. blattae* on the basis of size, nuclear structure, cysts, and the changes incident to karyokinesis. While little similarity was noted in *E. majestas*, *E. simulans* was found somewhat to resemble the amoeba of the cockroach in size and nature of cyst, in the arrangement and form of nucleoli, and in the changes

TABLE 1

Species	Size range and average	Nuclear size range and average	Nuclear shape	Nuclear membrane
<i>Endamoeba disparata</i> Kirby 1927	20-40 μ 32 μ	7.5-9 μ 8 μ	Spherical Fusiform	Delicate Distinct
<i>Endamoeba sabulosa</i> Kirby 1927	19-35 μ 26 μ	7-10 μ	Spherical	Delicate
<i>Endamoeba beaumonti</i> Kirby 1932	11-37 μ 17 μ	4.5-7 μ	Spherical	Delicate
<i>Entamoeba thomsoni</i> Lucas 1927	16-64 μ (living) 7-30 μ (fixed)	6 μ	Spherical	Distinct
<i>Endamoeba pellucida</i> n. sp.	14-36 μ 22.5 μ	7-11.5 μ 9 μ	Ellipsoidal	Thick
<i>Endamoeba blattae</i> (Bütschli 1878)	50-150 μ (adult) 50 μ	10-25 μ 15 μ	Spherical or slightly elongate	Very thick
<i>Endamoeba simulans</i> Kirby 1927	50-150 μ 77 μ	15-21 μ 17.6 μ	Spherical	"Definite but not unusually thick"
<i>Endamoeba majestas</i> Kirby 1927	65-165 μ 115 μ	15-26 μ 20 μ	Spherical	"Distinct but not especially heavy"
<i>Endamoeba lutea</i> n. sp.	34-151 μ 64 μ	6.5-17 μ 11 μ	Spherical	Definite but not thick
<i>Endamoeba granosa</i> n. sp.	16-97 μ 43.5 μ	5.5-20 μ 11.5 μ	Ellipsoidal	Definite but not thick
<i>Endolimax termitis</i> Kirby 1927	9.5-27 μ 16 μ	4-5 μ	Spherical	Delicate
<i>Endolimax blattae</i> Lucas 1927	3-15 μ	2-3 μ	Spherical	"Very fine but distinct"
<i>Endolimax suggrandis</i> n. sp.	11-48.5 μ 19 μ	3.5-5.5 μ 4.5 μ	Spherical	Delicate, indistinct

TABLE 1—(Continued)

Peripheral granules	Central granule	Other features of nucleus
Nucleoli against nuclear membrane	None	"Irregular granular mass of chromatin occupying a large part of the inner region"
Nucleoli but not against nuclear membrane	None	"Irregular mass of granular chromatin occupies most of central zone; several nucleoli at periphery of this"
"Scattered chromatic granules or a few large granules beneath membrane"	"Karyosomelike body"	"Large, granular, central chromatin mass"
Chromatic granules against nuclear membrane	"Central dot surrounded by a halo"	"Most commonly a large body of loose granules" in central zone
Chromatic granules against nuclear membrane	Central granule	Granular chromatin mass in central zone; shape roughly that of nucleus
Chromatic granules but not against nuclear membrane	"Dotlike karyosome sometimes"	Two zones: outer fibrous, with chromatic granules; inner densely reticular. Nucleoluslike bodies between them
None	"Karyosomelike mass"	Two zones: outer clear, inner dark. Chromatin in irregular karyosomelike mass in inner zone; small spherical nucleoli at periphery of this zone
None	Occasional	Irregular reticulum with many fine granules; aggregation of chromatic granules near center; irregular vacuolated masses of nucleolar material
Nucleoli occasionally against nuclear membrane	None	Granular reticulum, sometimes filling nucleus but usually contracted; two to nine large, rounded nucleoli
Plaques against nuclear membrane	Large spherical granule with halo	Irregular mass of large granules, often arranged in lines, in inner zone
"Deeply staining ring of peripheral chromatin"	Occasional	Large, irregularly outlined karyosome, apparently of plastin in which granules are embedded; radial filaments
"Little or no outer chromatin"	None	"Large central karyosome of achromatic groundwork with large chromatic granules embedded in it"; radial filaments not mentioned or shown
Granules against membrane, small and inconspicuous	None	Large central endosome (= karyosome), irregular outline; chromatin granules in achromatic ground substance; radial filaments

taking place during mitosis. These similarities led to the conclusion that the latter two forms were related, though possibly differing sufficiently to warrant subgeneric distinction.

It cannot be said that either of the two large amoebae of the present host shows a very marked similarity, other than in size range, to either *E. blattae* or the other amoebae of this group. In its nuclear shape and structure and in the nature of its cyst, *Endamoeba granosa* appears to stand alone. *E. lutea*, with its central reticulum and numerous nucleoli, presents a certain resemblance to *E. simulans* and, by the same token though less noticeably, to *E. blattae*. But there is a marked difference in the arrangement and size variation of the nucleoli, which appear to be peripheral in the latter two species and of fairly constant size, whereas in *E. lutea* they are scattered throughout the nucleus and show a great diversity of sizes. From what is at present known of the morphology of *Endamoeba granosa* and *E. lutea* it seems impossible to conclude that they present any very significant resemblance to other forms in this group.

The third group is, of course, separated from the other two by generic distinction. The resemblance and apparent close relationship of *Endolimax suggrandis* to *E. termitis* have already been observed. *Endolimax blattae*, which falls below them in size range, is more divergent but nevertheless appears to show close relationship to the other two. The endosome is of the same relative size as that of *E. termitis*, while the arrangement of chromatin within the endosome is more like that of *E. suggrandis*, being represented in two large-scale drawings as composing large particles more or less peripherally placed. Unlike the other two, *E. blattae* is reported to show frequent pseudopodia, to have a cytoplasm often filled with inclusions, and to occur rather frequently in binucleate form. The apparent absence of radial filaments is probably not significant, since in nuclei as small as those of *E. blattae* they would be most difficult to detect.

Morris (1936), reviewing the nuclear structure of the various amoebae now included in the genus *Endamoeba*, arrives at the following conclusion:

"They agree in having, during the mitotic interphase, an essentially concentric structure composed of a limiting membrane, a peripheral zone bearing granular chromatic material, an intermediate zone composed mainly of achromatic material but often containing granular chromatin, and a central point of focus at which a karyosomal dot can usually be seen at some time."

He recognizes three general types of nuclei (illustrated by diagrams) and proposes recognition of the following subgenera based on them:

(1) Subgenus *Endamoeba* for *E. (E.) blattae* Bütschli, 1878 (type) and other species having nuclear characteristics as follows: large nucleus invested by thick membrane; peripheral zone containing two distinct types of chromatic bodies; namely, numerous small, compact, darkly staining granules, and large nucleoluslike bodies; intermediate chromatic zone more pronounced than in other species and seldom exhibiting chromatic granules before the prophase; central karyosome, "seen only as the prophase approaches if at all," at the focal point of the achromatic island.

(2) Subgenus *Placoidea* for *E. (P.) minchini* Mackinnon, 1914 (type) and other species having the following nuclear characteristics: smaller nuclei (4 to 10 μ); comparatively thin nuclear membrane; peripheral chromatin habitually situated against the membrane in one or more relatively large plaques; rest of nucleus clear in living state but in fixed state containing a reticular central island which, during the interphase, appears to be composed of achromatic material bearing chromatic net knots and granules having a distribution markedly heavier toward the periphery than toward the center; dotlike karyosome usually apparent at the central focus of the reticulum.

(3) Subgenus *Poneramoeba* for *E. (P.) histolytica* Schaudinn, 1903 (type), and other species having the following nuclear characteristics: nuclear size and thickness of membrane approximately those of subgenus *Placoidea*. Peripheral chromatin composed of a series of comparatively uniform granules rather evenly distributed against the membrane; chromatin of the subperipheral region tending to be concentrated (by fixation) more heavily toward the center; a karyosomal dot usually visible at the focus of a more or less astrally arranged achromatic intermediate reticulum and frequently separated from it by a clear space.

On the basis of the criteria just given, no one of the present three species of the genus *Endamoeba* is easily placed. The subgenus *Poneramoeba* may be definitely excluded from consideration by the nature of the peripheral chromatin in that group, as well as by its size limitations where the two large amoebae are concerned.

Endamoeba pellucida conforms to the characteristics of the subgenus *Placoidea* in nuclear size, presence of peripheral chromatin against the nuclear membrane, and possession of a central mass of chromatin granules with a karyosome. It differs in having a noticeably thick nuclear membrane. Furthermore, while the granules of the central mass are evidently held by some sort of framework, the structure as a whole never presents the appearance of a reticulum, even when the granules are diffuse. If the proposed subgenera are accepted, *Endamoeba pellucida* should probably be assigned to the subgenus *Placoidia*, but it cannot be said that it conforms altogether to the characteristics outlined for that group.

Endamoeba granosa presents a similar problem. Its nuclear size would identify it with the subgenus *Endamoeba*, and its nuclear membrane is probably not so thin as to eliminate it from that group. In this species only one type of formed bodies is present in the peripheral zone, and these bodies are always against the nuclear membrane. The only other elements in the peripheral zone appear to be achromatic fibers, with only occasional small granules. The intermediate zone is a dense mass of large chromatic granules, and any achromatic structure present there is inconspicuous. In some ways the nucleus conforms more to the type of the subgenus *Placoidia*, but its size and the nature of its peripheral bodies, which do not stain like chromatin in Delafield's haematoxylin (pl. 62, fig. 22) would seem to eliminate it from that group. Assignment to the subgenus *Endamoeba* appears to be better justified.

Endamoeba lutea, like the species discussed above, conforms to the sub-

genus *Endamoeba* in nuclear size. Furthermore, it possesses large, nucleolus-like bodies and numerous small, compact, darkly staining granules. But these elements are not peripheral and to that extent do not conform to that type. However, this nucleus conforms still less to the characteristics of the *Placoidia* group.

In conclusion, a point made by Morris merits attention. He found numerous metacystic forms of *Endamoeba blattae* which appeared to be indistinguishable from *Entamoeba thomsoni* Lucas, thus raising a question of the validity of the latter species. He was unable to follow the life cycle from the *thomsoni* type of trophozoite to the adult form of *E. blattae*, but indirect evidence led him to suggest the possibility of origin of the adult by means of syngamic fusion of two of the smaller forms. The possibility of a similar relationship between *Endamoeba disparata* and *E. majestas* was further suggested, and indeed that other amoebae, from arthropod hosts, having nuclei similar to that of *Entamoeba thomsoni* might have a similar status. This would of course apply to *Endamoeba pellucida*. However, until experimental findings can establish the status of such forms, no other course seems indicated than to accord them specific distinction.

SUMMARY

1. Four new species of amoebae are described from a termite of the genus *Cubitermes*. They are:

a) *Endamoeba pellucida*, characterized by relatively clear cytoplasm with few ingested particles, an average size of $21 \times 22.5\mu$, and an ellipsoidal nucleus with a heavy membrane and a central mass of chromatic granules with a karyosome.

b) *Endamoeba granosa*, having a coarsely granular cytoplasm with abundant ingested material, an average size of $32.5 \times 43.5\mu$, and an ellipsoidal nucleus containing numerous granules and a conspicuous karyosome.

c) *Endamoeba lutea*, having a cytoplasm with numerous ingested particles, an average size of $48.5 \times 64\mu$, and a spherical nucleus occupied by a granular reticulum in which are dispersed deeply staining rounded bodies.

d) *Endolimax suggrandis*, with relatively clear cytoplasm, a typically elongated shape, an average size of $12.5 \times 19\mu$, and a spherical nucleus with a delicate membrane and a large endosome.

2. In addition, from the same host there are reported (but not described) a trichomonad flagellate, a smaller flagellate, and numerous bacteria and spirochaetes.

3. The nine previously described species of amoebae from termites and cockroaches are reviewed, and their affinities to the new species are discussed.

4. In considering the systematic position of the three new species of the genus *Endamoeba*, account is taken of a proposal by Morris of three subgenera for that genus. Attention is directed to certain difficulties in allocating the new species according to that plan.

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PLATES

All drawings were made with the aid of a camera lucida from material fixed in Schaudinn's fluid and stained with Delafield's haematoxylin (designated D.) or Heidenhain's iron-haematoxylin (designated H.). All drawings of whole specimens are to the same scale ($\times 1000$), and all drawings of nuclei are to a single larger scale ($\times 1600$).

PLATE 61

Figs. 1-10. *Endamoeba pellucida* n. sp.

Figs. 11-17. *Endolimax suggrandis* n. sp.

Figs. 1-2. Entire amoebae, showing vacuolated cytoplasm, distinct periplast, and nucleus with heavy membrane, peripheral granules, granular chromatin mass with karyosome, and peripheral fibers. H.

Fig. 3. Entire individual showing partly digested trichomonad in gastric vacuole. The nuclear granules are more diffuse. H.

Fig. 4. Nucleus showing central karyosomal granule and dispersed chromatic granules. H.

Figs. 5 and 8. Nuclei with compact granular masses. H.

Fig. 6. Nucleus in optical transverse section. The nuclear membrane is somewhat thicker than usual. H.

Fig. 7. Nucleus apparently lacking karyosomal granule. H.

Fig. 9. Spireme nucleus. Note persistence of peripheral granule. It was not possible to determine whether the thread is continuous or segmented. H.

Fig. 10. Nucleus of undetermined nature, perhaps an early prophase. Note that the peripheral granule is present. H.

Figs. 11-15. *Endolimax suggrandis*, showing vacuolated cytoplasm, nuclear structure, occasional ingested particles, and variety of shapes. Figs. 11, 12, 15—H. Figs. 13, 14—D.

Fig. 16. Nucleus, showing large irregular endosome containing achromatic ground substance and chromatic particles, radial filaments, and peripheral chromatin. D.

Fig. 17. Entire specimen with ingested filaments. The pseudopodium is exceptional. H.

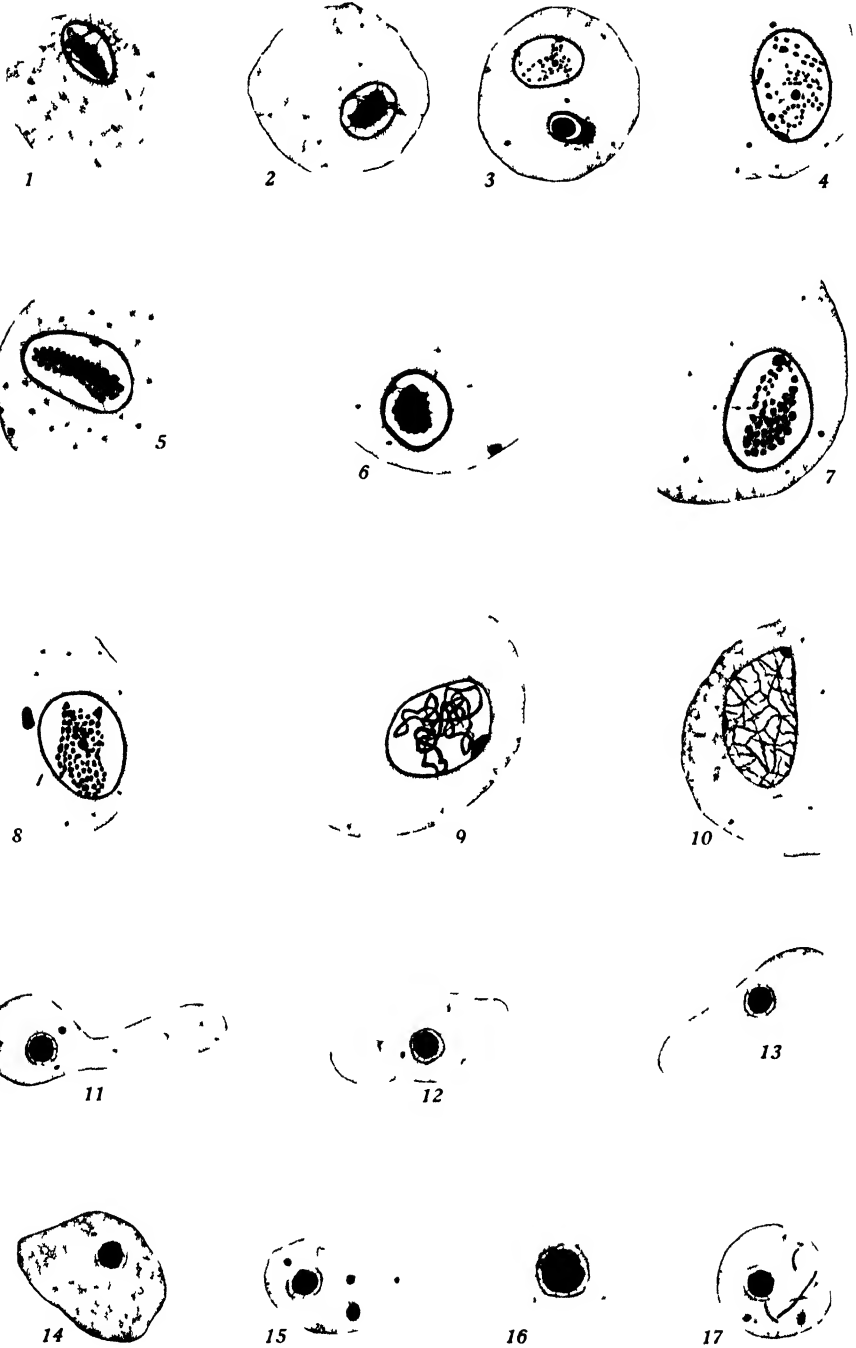


PLATE 62

Endamoeba granosa n. sp.

Fig. 18. Entire individual, showing coarse granules and ingested particles in endoplasm, projecting lobes of ectoplasm, nuclear membrane with plaques, and compact mass of granules in nucleus. II.

Fig. 19. Nucleus with granules, showing slight tendency to form threads; showing also karyosomal granule with halo and radial filaments, and heavy threads on nuclear membrane in place of the more usual plaques. II.

Fig. 20. Nucleus with mass of small granules. II.

Fig. 21. Nucleus with granules tending to be diffuse. The peripheral plaque appears to be composed of more than one substance. II.

Fig. 22. Nucleus in which the granules are definitely arranged as threads. Note the light staining of the peripheral plaques. D.

Fig. 23. Cyst. Only three nuclei can be seen. II.

Fig. 24. A small form. The nucleus is too deeply stained to show granules. II.

Fig. 25. Individual of a type characterized by wide zone of ectoplasm, few ingested particles, and nucleus with fusiform mass of tightly packed threads of granules. II.

Fig. 26. A larger form. All granules in the nucleus were stained to the same degree, the lighter ones being at a lower optical level than the dark ones. II.

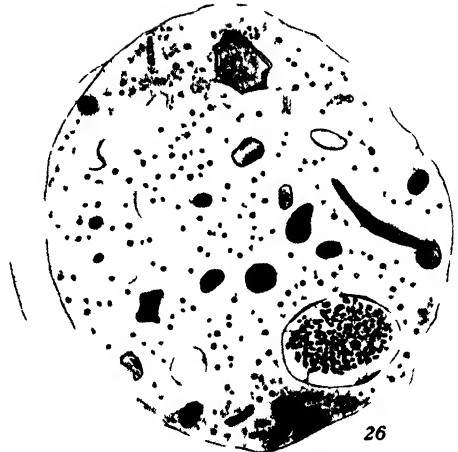
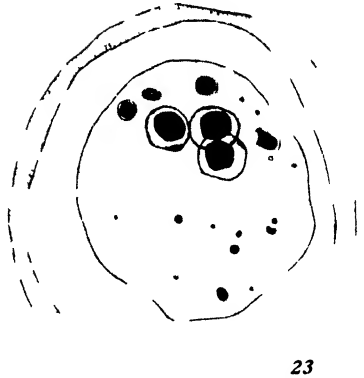
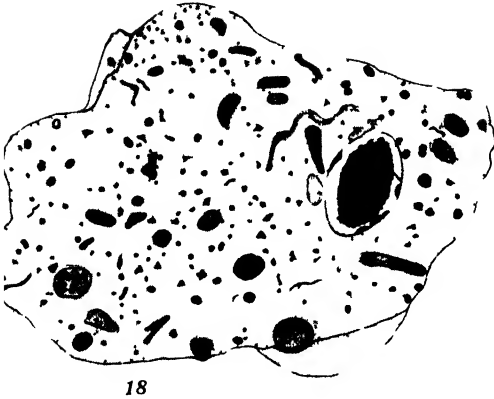


PLATE 63

Endamoeba lutea n. sp.

Fig. 27. Small amoeba, showing granular cytoplasm, narrow lobe of ectoplasm, ingested particles, and nucleus with reticulum and nucleoli. H.

Fig. 28. Larger form, showing numerous large ingested particles, nucleus with contracted reticulum, and tendency of granules to form threads. The lighter nucleoli are stained to the same degree as the darker ones but are represented as being at a lower optical level. H.

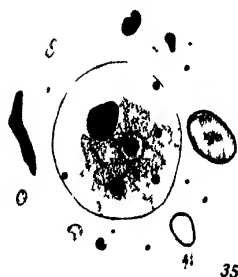
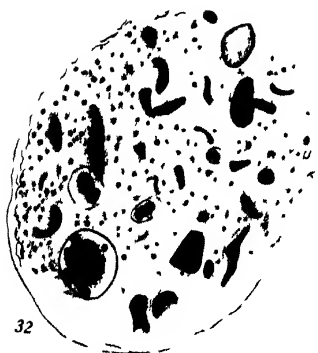
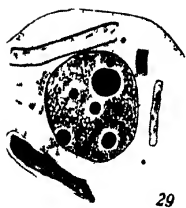
Fig. 29. Nucleus with reticulum occupying the entire volume. The large nucleolus is a common feature of nuclei of this species. II.

Fig. 30. Nucleus of the type shown in fig. 27, drawn to a larger scale. II.

Figs. 31, 33, 34. Nuclei showing variety of patterns. H.

Fig. 32. Entire amoeba of compact form, showing narrow rim of ectoplasm with broad zone at the bottom. Note that ingested particles are not confined to the endoplasm. II.

Fig. 35. Large nucleus, showing characteristic large nucleolus, which is irregular in shape and unevenly stained. Note the delicate net of granules in the clear zone and the tendency of granules in the reticulum to form a netlike pattern. II.



A STUDY OF OXYMONAS MINOR ZELIFF
FROM THE TERMITE
KALOTERMES MINOR HAGEN

BY

JOY BARNES CROSS

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A STUDY OF OXYMONAS MINOR ZELIFF FROM THE TERMITE KALOTERMES MINOR HAGEN

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INTRODUCTION

SINCE JANICKI described *Oxymonas granulosa* from *Neotermes connexus* in 1915, seventeen additional species of *Oxymonas* have been reported, all having been found in the intestines of termites of the family Kalotermitidae. This specificity of hosts is of evolutionary significance, as are also the indications of a series of intergrading structural and functional forms within the Oxymonadinae (Kirby, 1937). A study of the functional pattern has another generally significant correlation with the problem of protozoan reinfection of new colonies of termites because of the characteristically firm attachment of *Oxymonas* to the chitinous intima that lines the intestinal tract.

Although *O. minor* is one of the smaller and less complex members of the group, it is by no means lacking in interest when considered in connection with these general values, for it reflects the variability seen within the genus in its size, in its form, and in the number of its nuclei.*

O. minor occurs in the intestine of *Kalotermes minor* Hagen and was reported by Zelif in 1930 with the following description :

Oxymonas minor sp. nov. . . . The species of *Oxymonas* from *K. minor* Hagen from California, differs [varies] greatly in size. The average size of 100 is 13 by 25 μ . The nucleus is typically elongate and has an average size of 4 by 7 μ . The karyosome and halo have diameters of 1 and 2 μ respectively. The rostellum is very variable in length; and one with a length of 105 μ is present. The long rostellae are very narrow. The blepharoplasts are spherical and the flagella are about twice the length of the body of the organism. The organisms contain wood in various amounts. The axostyle may project beyond the posterior end of the body and in individuals containing several nuclei a corresponding number of axostyles may be present. The size of the organism, the type of the rostellum and nucleus are characteristic.

At this same time Zelif published a comparative study of all the previously described species of *Oxymonas* and added eleven new species. Cleveland (1935) briefly summarized all preceding reports with his description of *O. grandis* and questioned the validity of the additions made by Zelif, protesting the establishment of a different species of protozoan solely on the basis of its existence in a different species of termite.

In 1930 Kirby suggested that Duboseq and Grassé's "avicular form" of *Trimitus* from *Kalotermes flavicollis* very much resembled what appeared to be developmental stages of a small *Oxymonas* seen in *K. minor* and in *K.*

* I am sincerely grateful to Dr. Harold Kirby for suggesting this study and for his valuable advice and criticism.

hubbardi. In 1934 Duboscq and Grassé further described the animal and established a new genus for it with the following definition:

Opisthomitus n. g. Polymastigine mono-ou polyénergide. L'individu uninuclé porte quatre flagelles égaux, rebroussés en arrière, le plus souvent rabattus sur un même côté du corps et partant ordinairement de deux blépharoplastes unis par une desmose. Rostre sans fibre rétractile; noyau à petit caryosome; appareil parabasal typique; un axostyle. Nutrition par osmose. Pas de vêtture schizophtyque.

Except for the parabasal apparatus, the figures and description of *Opisthomitus* fit the appearance of small forms of *Oxymonas minor*. Large granules were occasionally seen after the use of Flemming's fixative in much the same position as indicated by Duboscq and Grassé for the parabasal of *Opisthomitus*. Kirby (1928) reported similarly for *Proboscidiella*. It was suspected that the presence of the granules was correlated with the absence of rostellar fibrils; but their appearance proved inconstant, and it was decided that they represented merely another aspect of the deeply staining granules which occur frequently in *Oxymonas minor* with no apparent significance.

Duboscq and Grassé have further opposed the suggested synonymy by claiming a distinction for *Opisthomitus* because of the possession of a clear rostellar peak and the lack of rostellar fibrils. Attention is directed to a similar condition in *Oxymonas minor* (pl. 65, fig. 9). Although the specimen represented by this figure is approaching metaphase, its neighbor (pl. 65, fig. 8) is so nearly in the same mitotic period as to minimize the explanation of the lack of fibrils on the score of mitotic change. Connell (1930) also reported this reversal in the staining reaction of the rostellar peak in the motile form of *O. dimorpha*, but Duboscq and Grassé seem to have overlooked his statement.

Kirby (1937), following Cleveland (1934) with slight alteration, placed *Oxymonas*, *Proboscidiella*, *Microrhopalodina*, and *Kirbyella* in the family Pyrsonymphidae, subfamily Oxymonadinae. The related subfamilies of Pyrsonymphidae are Saccinobacculinae, with one genus, *Saccinobacculus*, and Pyrsonymphinae with two genera, *Pyrsonympha* and *Dinenympha*.

MATERIALS AND METHODS

The termites were taken from the wood and placed in a moist chamber with two sheets of filter paper and some of the wood. The filter paper was kept very slightly moist, for, although *Kalotermes minor* is known as a "dry-wood" termite, excessive dryness is quite as fatal as excessive moisture.

In the making of smears, the extracted gut was crushed and the contents diluted slightly with 0.6 per cent NaCl. Cold Schaudinn's fixative, followed by Heidenhain's or Delafield's haematoxylin, was used for the most part. Zelif's (1930) suggestion that Flemming's fixative, followed by overstaining with iron haematoxylin, was excellent for observation of the blepharoplasts proved valuable. This fixative also showed a clearer distinction between the intensely staining cytoplasmic granules and the inclusions commonly called "spherules."

Destaining was practiced in some instances much beyond the customary

procedure, because Cleveland (1934) had questioned Connell's statements with respect to the origin of the centrodosome from the karyosome on the score that the latter had been overstained. Since the nucleus is so very small (its structural details require magnification by at least an 8× eyepiece and 100× objective), the destaining was estimated by the appearance of larger animals. Overstaining was considerable in *Staurojoenina* and slight in *Metadevescovina*.

Giemsa stain proved to be of no value whatever. Lugol's solution was used and permitted many of the same observations that were later seen with greater definiteness after the use of Flemming's fixative and Heidenhain's haematoxylin stain.

Connell (1930) reported having obtained an almost pure culture of *O. dimorpha* after feeding the termites cane sugar. A method of increasing the percentage of *O. minor*, particularly for the study of living material, seemed highly desirable.

A thorough test could not be made because of the inadequate supply of the host termite. In July, animals fed on sugar for three days gave smears that were possibly a little improvement on the average. Of the smears from six animals fed for four days, two slides were extraordinary for both size and abundance of *Oxymonas*. Among six animals kept for five days on cane sugar, there were only two feeble survivors. These supplied smears of but slight value. In November, six termites survived a fourteen-day feeding. It is suspected that the lessened humidity within the covered containers during the cooler month accounted for the greater viability. The smears showed a paucity of protozoa, but the majority of these were *Oxymonas*.

In an attempt to secure division stages by the production of "mitotic flares," two groups of animals were examined: (1) a group which had been fed on filter paper for four and five days, respectively, and (2) newly moulted animals (Andrew and Light, 1929). In the latter group were included individuals with little body coloration, and a few with none. These last were used because Connell (1930) had noted that *O. dimorpha* occurred during refaunation at an earlier period than did any other protozoan. Slides were labeled either "ecdysis" or "extreme ecdysis."

Such highly variable results were obtained that none of the methods tried could be considered certainly superior to the others.

Smears were also made from alates. Those marked "early" were from animals fixed in the morning after having been extracted from the colony the previous evening. The "24-hour" smears were from specimens fixed 24 hours later, and the "48-hour" from specimens fixed 48 hours later. These served as checks against serial sections cut from the intestines of animals classified in the same fashion.

Since the intestine was much reduced in size and the contents seemed more fluid than in that of nymphs and had a greater propensity for washing from the slide in the fixative, three animals were used to each smear. *Staurojoenina*, *Metadevescovina*, and *Oxymonas* occurred in all smears, though sparsely.

The serial sections of the intestine were made from animals taken from wood. The gut was extracted just as in making smears, fixed in Bouin's fluid, embedded in paraffin, cut at 9μ , stained with either Heidenhain's or Delafield's haematoxylin, and counterstained with eosin at pH 5.4–5.6 (Galigher, 1934).

The tissues of the gut wall stained excellently, but the staining of the protozoans was less satisfactory than in smears. It was presumed that the chitinous intima lining the gut interfered with the action of the fixative.

To insure penetration of the gut lumen by the fixative, the intestine was extracted, dropped into Bouin's fixative, and then, being held vertically above the fluid, was cut with fine scissors perpendicularly to the long axis through the vestibule and dropped quickly back into the solution. Observations showed that the regions nearest the cut had few or no *Oxymonas* and few or no inhabitants at the center of the lumen. At a slightly greater distance, although the central contents were still much reduced, the number of *Oxymonas* along the gut wall seemed normal and they stained well.

It was also noticed that *Oxymonas* stained well in alates in which the central region of the lumen was little crowded and free of debris, probably the result of suspended feeding during the moult (Andrew, 1930). This freedom from debris, together with the frailty of the gut itself at this period, suggests that the study of living *O. minor* might be facilitated by using the winged form of host.

More recently, at the suggestion of Dr. K. De Ome, intestines fixed in Schaudinn's, Hollande's, or Flemming's solutions have been cleared with tertiary butyl alcohol and have made very satisfactory sections.

In destaining the sections, *Staurojoenina* could not be used as a gauge. With the exception of the nucleus, it lost haematoxylin much more rapidly than did *Oxymonas*, a reversal of the behavior of this stain on smears after fixation with Schaudinn's or Flemming's solution.

Delafield's stain followed by eosin showed the structure of the intima better than did Heidenhain's haematoxylin. Although the axostyle does not stain with Delafield's haematoxylin, as Kirby (1928) has already noted, it takes enough eosin to make its position distinct. The centrodosome also stains with eosin, though the outermost edges of the band show a little of the effect of haematoxylin.

For the study of living animals, the intestine was extracted, placed in the depression of a hanging-drop slide, crushed, and diluted with a little 0.6 per cent NaCl. If a coverglass sealed with vaseline was added and light was excluded, portions of this might be removed to other slides from time to time over a period of about four hours and further diluted with the NaCl to produce a concentration of the animals suitable for observation.

LOCALIZATION IN HOST

Although *Oxymonas* appeared in smears in a ratio of about 1 to 4 per cent of the total number of protozoans, it is apparent that, in sections from the intestine of nymphs, the proportion exceeds this. The three major inhabitants

of the gut as listed by Kirby (1934), *Staurojoenina assimilis*, *Metadevescovina cuspidis*, and *Oxymonas minor*, were usually disposed in concentric zones. *O. minor* occurred in a band of relatively constant width about the periphery of the lumen, *Staurojoenina* was found in a circular band immediately inward from the region occupied by *O. minor*, and *Metadevescovina* filled the central region. Where the lumen is small, *Metadevescovina* may be almost entirely lacking. It should be noted further that the bandings of *O. minor* were wider between infoldings of the gut wall. Once, in observations on living *Oxymonas*, dozens of the form represented in plate 65, figure 9, were seen crowded in attachment to a fragment of gut, duplicating the situation found in stained sections. A definite orientation of the anterior end of the animal toward the gut was maintained with much jostling and turmoil. Displacements occurred and reattachments were attempted, giving the semblance of conflict for position. Child (MS) has given similar description of the behavior of *Streblomastix* in *Zoötermopsis nevadensis*, and has suggested a thigmotactic tropism. Protozoans were found almost exclusively in the vestibule, caecum, and the ascending and descending limbs of the large intestine (Child, 1934).

Considering the apparent disparity between the number of *Oxymonas* found in smears and those found in sections, it seemed of value to compute the relative numbers of *O. minor* and the other protozoan inhabitants of *Kaloterms minor*. Since sectioning mutilates many animals, the nuclei were made the basis of the count. Uncrumpled sections from various regions of the gut, all cut 9μ thick, served as units in sampling. The locations of these sections in the gut were identified according to the terminology used by Child (1934) in his study on *Z. nevadensis*.

Hairlines were placed in the eyepiece of the microscope to make a squared field for counting. This squared field lessened the danger of omitting certain areas in shifting to a new field or of double counting others. There still remained the possibility, in regions crowded with nuclei, that some of these might be omitted or double counted. However, this would not introduce a large error and, since chance would operate equally against both groups, it should not disturb the ratio between them. Because of their comparatively rare occurrence, the possible presence of multinucleates was disregarded. The counts are shown in table 1.

These figures convey two significant facts. Even the lowest percentage of *Oxymonas* in the serial sections far exceeds the range of 1 to 4 per cent found in many smears. Apparently *Oxymonas* adheres firmly to the gut wall; consequently most of the individuals are discarded along with it when smears are made.

The percentage of *Oxymonas* varies inversely with the size of the lumen, the smaller percentages coming from the widest portions of the gut, where there are large numbers of other protozoa, *Metadevescovina* in particular. Therefore, although the peripheral banding of the lumen by *Oxymonas* remains relatively constant in width throughout the protozoan-inhabited region of the intestine, the percentage of *O. minor* increases extravagantly where the

lumen is very small, simply because the percentage of *Metadevescovina* approaches zero.

Thus, the variability in the distribution of *O. minor*, as indicated by the range of 30.6 to 85 per cent, is more apparent than real, being actually an inverse measure of the number of other protozoans in the given region.

O. minor occurs in crowded masses, often seven or eight animals deep, packed tightly against the intima lining the vestibule, caecum, and both limbs of the large intestine. In some instances small animals are so compactly massed as to give the intima a cellular appearance. Extreme variation in size occurs, sometimes in closely associated animals (pl. 64, fig. 1); but, in general, near-by regions will consist of very large or very small or, more commonly, of elongate

TABLE 1
PERCENTAGES OF OXYMONAS IN GUT OF KALOTERMES MINOR
AS DETERMINED BY NUCLEAR COUNTS

Region of Intestine*	Total number of Nuclei	Number of Nuclei of Oxymonas	Percentage of Oxymonas
Ascending limb.....	269	177	67.0
Vestibule, just posterior of central region.....	2728	835	30.6
Descending limb.....	239	187	78.0
Posterior vestibule.....	899	719	79.8
Posterior vestibule.....	865	735	85.0
Posterior vestibule.....	1381	1044	75.0
Central vestibule (probably portion of caecum also).....	3402	1301	38.0
Anterior vestibule.....	1617	1169	72.0
Vestibule, just anterior of central region.....	3573	2645	74.0
Vestibule, just posterior of central region.....	2178	703	32.0

* Sections were taken from five different animals.

intermediate forms. From this it would seem that the conditions that stimulate division tend to operate synchronously upon the animals in any one neighborhood.

Judging by these group studies the presence or absence of an extended rostellum seems to be dependent on the animal's proximity to the gut wall. However, rostella have been seen on individuals of the free, small, spherical type that is most frequently found in the lumen (pl. 65, figs. 8, 11); in rare instances, an extended rostellum has been seen also on a free, large animal.

The small rounded forms seen in sections accompanying the large, easily recognizable *Oxymonas* might be considered to be only portions of animals cut in making the paraffin ribbon; but identical forms and nuclei are seen in smears as well. Further, these numerous small forms are too uniform in appearance to be chance products of cutting.

In one group these flagellates ranged from 5 to 25 μ in length. Ordinarily, the small forms do not show an extension of the rostella in the attached stage, but pack in closely against the intima between the rounded anterior

regions of the larger animals. The chromatin in their nuclei usually shows as a smooth black line at the periphery, with only a few faint strands between it and the centrally located karyosome, which may stain more faintly than it ordinarily does in the larger animals. Such nuclei are interpreted as having recently undergone division (pl. 64, fig. 1; pl. 66, fig. 18). In this stage the nucleus very much resembles the structure in Kofoid and Swezy's figure of *O. projector* labeled, "Optical cross section of body showing retractor fibers in sleeve" (1926).

To determine whether the rostellum passed the limits of the intima and made attachment to the underlying epithelium a few animals were examined in which the intima was retracted from the epithelium in preparation for ecdysis. In no instance was a rostellum seen protruding beyond the intima and all the evidence indicates that it never penetrates beyond the base line.

Child (MS) has reported that, in the last ecdysis of the reproductive caste, unlike the earlier moults, the old, shrunken intima retains some of its protozoa and that these later break through the thin wall to refaunate the gut of the adult. This study tends to corroborate his statement.

Judging from the maturity of egg cells found on one slide, the termite (nymph) must have been very close indeed to the last ecdysis. In one region the intima was contracted into a narrow, sacklike state, and contained *Staurojoenina*, *Metadevescovina*, and *Oxymonas*.

In sections made from alates there were further indications that Child's explanation of alate refaunation in *Zoötermopsis* was also true for *Kaloterms minor*. In several sections from one area the old intima could clearly be seen as a crumpled veil about small forms of *Oxymonas* and *Metadevescovina*. The new intima was well developed along the epithelium in this region, and some animals lay free in the lumen.

Child has stated that the alates feed sparingly before flight. This was also noted in winged animals segregated from the colony. The ejected wood pellets, however, differed in color from those in nymph-inhabited colonies.

The classification of alates into "early," "24-hour," and "48-hour" did not prove sufficiently precise; Child's (MS) scheme, based on the condition of the wings and on the body coloration, should prove more satisfactory. This lack of precision in classification probably accounts for the failure to observe any distinction among the three groups in the relative numbers of *Staurojoenina*, *Metadevescovina*, and *O. minor*. In some sections only a few animals were present; in others, the lumen, though much reduced in size, was well filled. There was little debris. Many prophase stages of nuclei were recognized. No counts were made, but inspection suggested little variation from the percentages established for the nymph gut.

Because of the close association of *O. minor* with the gut intima, this chitinous derivative of the termite epithelium assumes importance. Child (MS) states that its thickness varies from 1 to 4 μ . In plate 64, figure 1, the intima is retracted from the epithelial cells and is shown as a dusky line of varying width along the left border of the crowded mass of *Oxymonas*. The

strandlike extensions of the intima are not fully sketched. Actually, they still bridge the gap between the intima and the epithelium. This observation was made on a seventh-instar female which was in preparation for the last moult.

LIFE CYCLE

Prophase nuclei were abundant in the many small, subspherical *Oxymonas* seen within the old crumpled intima retained in the intestine of an "early alate." Division of the animals, which seemed imminent, would have produced the spheroid type usually seen free in the lumen. Since these are also seen in the attached stage, it seems logical to suppose that migration and then anchorage follow division. In conformity with Connell's (1930) report for the motile type of *O. dimorpha*, the small forms of *O. minor* lack inclusions. Feeding and growth presumably occur only after attachment and result in intermediate forms and eventually in the largest, more or less elliptical, stage.

It is unknown whether full size must be attained before a series of mitoses can convert the large forms into the small spheroids. It is known that mitosis occurs in the smaller forms; and it seems probable that, whenever the physiologic state of the gut favors division, mitosis occurs, unless the animal has already reached its minimum. It also seems likely that division would take place whenever an animal has achieved maximum growth.

Connell (1930) reported that division ceased with the loss of "volutin," the term he employed for the light-brown spherules mentioned by Janicki (1915), Kirby (1928), and Zelif (1930). Contrary to this report, division stages were seen in smaller animals than those which show spherules.

If, as Child (MS) has postulated and our observations indicate, refaunation of alates is accomplished by a retention of protozoans within the sack of the old intima, there must be during the last ecdysis either a marked destruction of the large *Oxymonas* or a rapid conversion into the small forms, which seem to be the sole survivors of that period. May (1941) has confirmed the retention throughout ecdysis of certain protozoa in the intestine of *Zoötermopsis nevadensis* and of *Kalotermes minor*. Kirby (1930) noted the persistence of *Tricercomitus* in *K. minor* and has briefly summarized the various reports (1941).

No observations were made with respect to the manner of refaunation in nymphs; presumably, it results from proctodeal feeding (Andrew, 1930; Child, MS). Observations on the life cycle of *O. minor* in the nymph gut between the periods of ecdysis approximate those described for the alates.

Since ecdysis has been considered an unfavorable physiologic state for the intestinal protozoa, the production of the small form at that time indicates that it is the more resistant type. Conversely, we might suppose, the large forms argue a favorable environment. Possibly, the presence of multinucleate forms indicates a variability in the physiologic state of the gut. Disadvantageous conditions initiate nuclear division; but when a favorable state intervenes, growth is resumed and cytoplasmic division is postponed. Connell (1930) postulated that delayed plasmotomy resulted in the multinucleate animals.

GENERAL MORPHOLOGY

As indicated by the groupings of *O. minor* along the intestinal wall, the shape and size are highly variable, ranging from large, somewhat elliptical animals to small spheroids. For purposes of measurement, it seemed wise to recognize two classes (the smallest spheroids and the largest elliptical type), to determine their averages, and to let these define the size range. All measurements were made on smears.

In 25 spherical animals, chosen from two slides, the average diameter was 7.3μ , the minimum 5.8μ , and the maximum 10.0μ . In 10 nuclei, the average diameter was 3μ , and the range inconsequential.

In 25 of the large ellipsoid animals, the average length exclusive of the rostellum was 24.7μ . The minimum is valueless, since it was arbitrarily chosen and merges with the intermediate forms. The maximum was 30.8μ . One monster was seen, possessing 5 nuclei and measuring 81μ . The average width was 13.2μ . In general the width approximated half the length of the animal. For 10 nuclei, the average measurements were $6.3 \times 4.7\mu$.

O. minor has an average size range of 7.3 – 24.7μ , and an extreme size range of 5.8 – 30.8μ . Almost every variation in size and shape possible between these two extremes is represented in the intermediate forms.

The anterior end of the animal may be extended into a long, narrow organelle of attachment or it may exist as a clear, peaked mound. The rostellum is not infrequently more than three times the body length. It has been seen extended on all types of *O. minor*, but it occurs less frequently on the smaller forms.

Satisfactory observations of the holdfast, the attachment organelle, were not possible, and it can only be stated that some modification of the rostellar tip is evidenced. The central core of the rostellum is clear. The rostellum usually appears cylindrical in form, but occasionally it is ribbonlike (pl. 65, fig. 6). Two strands of deeply stained fibers may often be traced almost to its end. These originate, at least in part, from the inner granules of the pair of blepharoplasts situated on either side of the base of the rostellum. Each blepharoplast consists of two very closely joined granules. Except after use of Flemming's fixative, these appeared as single, small, plump rods with bulbous ends.

Each of the exterior granules in both pairs of blepharoplasts supports two flagella (Kirby, 1928; Zelif, 1930; Connell, 1930). The length of these is not easily determined in smears because of the fibrous nature of the surrounding debris, but it considerably exceeds the body length. This is indicated by a specimen in which the length of the body was 25.7μ and that of the flagella 34.8μ , and by the measurements taken from the illustration of *O. minor* as seen with dark-field illumination (Kirby, 1928), in which the length of the body was 22μ , that of the flagella 36μ .

The inner granules are joined by a connecting fibril in addition to the anterior rostellar fibrils, and each sends a short rhizoplast toward the nucleus,

"terminating in what appears in many instances to be a small body lying in or on the nuclear membrane. . . . It is possible that these small bodies are centrosomes" (Zeliff, 1930). One of the inner granules joins the blunt end of a portion of the axostyle. Sometimes the other granule supports a posterior cytoplasmic fibril (pl. 64, fig. 2).

The remainder of the rodlike axostyle extends anteriorly into the rostellum as a fibril. Sometimes the proximal end of the axostyle gradually widens from a point a short distance below the nucleus (pl. 64, fig. 4; pl. 65, figs. 6, 8). It stains intensely with Heidenhain's haematoxylin but refuses Delafield's haematoxylin (Kirby, 1928). Usually it protrudes slightly beyond the body and carries a slim tip of cytoplasm with it. A distal ring encircles it at the point of exit. Occasionally an arrowhead tip is seen (Kirby, 1928). In plate 64, figure 2, this is shown distinctly, but ordinarily the tip did not extend as far from the body. With Lugol's stain, and after fixation in Flemming's solution, the proximal portion of the axostyle appeared broader, showing a little of the character of the axostyle of *O. grandis* (Cleveland, 1935). Its position is definitely excentric in the animal's body.

The cytoplasm is finely granular. Often, dark-staining granules are seen (pl. 64, fig. 3). Zeliff (1930) notes these as extruded chromatin or chromidia, but he makes no mention of the results of a Feulgen reaction. Light-brown spherules, mentioned by Janicki (1915), Kirby (1928), Zeliff (1930), and Connell (1930), are also present. Plate 65, figure 13 shows them in an attached animal. Kirby agreed with Janicki that these spherules were probably derived from ingested wood. Connell called them "volutin." Wood particles may frequently be distinguished among the body inclusions. Vacuoles are not infrequent.

The pellicle appears firm, but rodlets like those seen on *O. grandis* were not distinguishable. Body deformation occurs much less frequently in *O. minor* than in *O. grandis*. Janicki (1915) reported a similar discrepancy in the morphology of *Stephanonympha major* and of *S. minor*.

The interkinetic nucleus is situated anteriorly at the base of the rostellum and is subovoid to subellipsoid. Chromatin granules outline the periphery. A karyosome, surrounded by a halo, lies slightly posterior of the central region. A few faint strands cross the halo and connect with the irregular reticulum of coarse granules that loosely fill the remainder of the nucleus (pl. 64, fig. 2).

When favorably stained, the rhizoplast attachment granule near the anterior border of the nucleus shows distinctly. Sometimes a very small halo appears around it. Zeliff (1930) considered that this might be the centrosome, and these observations support his opinion.

The inconstant occurrence of what was called a "sleeve" by Kofoid and Swezy (1926) has been noted in *O. minor*, but it is not considered an organelle. Rarely, the axostyle passes across it. Focusing indicates that the deeply-staining structure shown in the cytoplasm of plate 64, figure 2, is ribbonlike, not cylindrical, although otherwise it seems to correspond to Kofoid and Swezy's "vestigial sleeve."

MITOSIS

In 1930 Zelif outlined a general scheme of mitosis for *Oxymonas*. Briefly, it consists of: (1) a prophase in which the nucleus usually migrates to the posterior region of the cell and the karyosome elongates to produce the centrodesmose; (2) a metaphase in which a girdle of chromatin rather than an equatorial plate forms and in which the centrodesmose is clearly seen; (3) an anaphase in which the strands of chromatin move to the poles of a conspicuous centrodesmose; and (4) a telophase in which the nuclear membrane constricts to form the two daughter nuclei.

Connell (1930) confirmed the formation of the centrodesmose from the karyosome (Janicki, 1915; Zelif, 1930) in *O. dimorpha*. Cleveland (1934) criticized this on the score that Connell's material seemed to have been overstained, and in 1935 described the achromatic mitotic figure of *O. grandis*, in which he reported the absence of a karyosome. The present study indicates that in *O. minor* the karyosome does not form the centrodesmose.

Nuclei with two karyosomes were observed and figured by Janicki (1915) in *O. granulosa* and by Zelif (1930) in other species. Zelif postulated that such nuclei indicated either an amitotic division or a modified mitosis. My observations support the latter method.

No indications of a posteriorly migrating prophase nucleus were found. Yet it is usually expected that prophase figures will be present in larger numbers than the other mitotic stages. Janicki's figures (1915) indicated this same lack in *O. granulosa*. Instead, there was the persistent recurrence of nuclei having the characteristics illustrated in figures 3-9, plates 64 and 65. Significance was implied by constant repetition. Because of the lack of nuclei conforming to the accepted prophase type and because of the constant repetition of these atypical forms, which apparently form a logical series of steps between the interkinetic nucleus and the metaphase girdle, these forms are considered as possible stages in the prophase of a modified mitosis in which the duplication of the karyosome is an essential step.

The series is initiated by a curious downward bulge of the posterior membrane of the nucleus. There is some shifting in position of the chromatin granules, but no definite pattern has been discovered. The halo about the karyosome seems to enlarge. The posterior membrane is very delicate and the region which it encloses remains practically clear (pl. 64, fig. 3).

The granules are next arranged in double strands running in an anterior-posterior direction across the karyosome, but they do not enter the clear space in the posterior end of the nucleus (pl. 64, fig. 4). The strands consist of chromomeres in a matrix of slightly lighter-staining material. Except for its elongation, the nucleus is very similar to Zelif's (1930) prophase nucleus.

A diffuse state follows, but only in that portion of the nucleus formerly occupied by the chromatin strands. At this stage, the centrodesmose may be distinguished (pl. 65, fig. 5). However, earlier than this, fibrils are present which might easily be interpreted as centrodesmose, were it not almost im-

possible to differentiate them certainly from the chromatin strands which are also in formation at that period. From the activity within the nucleus it would appear that mitotic elements had formed even earlier.

The centrodosome seems to originate within the nucleus from the region occupied by the attachment granule which was interpreted by Zelif (1930) as a centrosome. Possibly, *O. minor* also exemplifies the condition described by Cleveland (1935) for *O. grandis*, in which there is an achromatic producing portion of the centriole and a portion from which extranuclear organelles develop.

The karyosome elongates in an axis perpendicular to the long axis of the nucleus. There are often vestiges of the earlier strands in scattered, paired granules (pl. 65, fig. 6). It is believed that these are granules similar to those which Janicki (1915) figured (Taf. XVII), which for a time he suspected as the source of the centrodosome. Eventually, because of their similar staining reactions, he considered that the centrodosome more probably originated from the karyosome.

The karyosome divides in an anterior-posterior line and sometimes a portion lies on either side of the centrodosome (pl. 65, fig. 7). This is easily distinguishable since the chromatin, which now fills the entire nucleus, is still diffused. Small peripheral granules have again appeared. One of the daughter karyosomes moves anteriorly from the equator, and the other moves posteriorly. The chromatin again becomes granular, and a patternless reticulum forms with a halo around each of the two new karyosomes (pl. 65, fig. 8). The granules increase in size and become more chromophilic. They drift toward the equator between the two karyosomes (pl. 65, fig. 9), and form the typical metaphase girdle (pl. 65, fig. 10).

With the metaphase the nucleus starts its posterior migration and the axostyle begins to degenerate. However, because the specimen represented by figure 10 (pl. 65), is stained with Delafield's haematoxylin, the axostyle does not disappear as quickly and completely as the figure seems to indicate. This may be seen by comparison with figures 11 and 12 (pl. 65), in which the nuclei, although representing later stages, still show traces of this organelle.

Throughout the prophase, there is some slight intimation of a nuclear border of clear, undifferentiated material. In metaphase, the region immediately surrounding the nucleus is sharply outlined. There is much less definite connection of this clear material with the rostellar region of the animal.

The nuclear membrane is finely granular and is indented at the ends where the centrodosome touches it. From either end of the centrodosome, short, faint fibrils are seen passing into the clear nuclear border. Large granules of chromatin are arranged in linear rows to form a hollow cylinder about the centrodosome. In many of the metaphase stages, the girdle appears broader and the granular structure of the chromatin cannot be recognized. The karyosomes disappear. They are not again in evidence until the tightly clumped, dark chromatin mass becomes diffuse in a late reorganization stage (pl. 66, fig. 17).

It is assumed that the early anaphase passes very swiftly, since no very satisfactory stages of it were obtained. Plate 65, figure 11, illustrates the beginning of movement of chromatin from the metaphase girdle toward the poles, but heavy stain obscures the structure. The nucleus has tilted a little. Tilting must necessarily take place in the early anaphase to provide for the change in orientation between the metaphase and the late anaphase. In the latter period, the nucleus lies with its long axis perpendicular to its former position during the metaphase (pl. 65, fig. 12).

The nuclear membrane is still indented at the poles. The chromatin appears in short strands of granules forming a "tassement polaire" at either end of the centrodosome, which seems to have a somewhat lighter-staining core. The nuclear border of clear material is slightly less well defined in this figure, but there are still indications that it maintains a connection with the rostellar region.

A small fibril is seen to extend from the tip of the centrodosome at the upper pole. It ends in a granule on the periphery of the nuclear border material where it connects with a delicate, short fibril extending perpendicularly from it on either side. This is presumed to be the new axostyle. No fibril could of a certainty be recognized at the lower pole. With the exception of the distal ring, the axostyle has been largely resorbed.

In the telophase (pl. 65, fig. 13), the daughter nuclei have separated. They are still joined by the centrodosome, which has dwindled from the stout, straight band in the metaphase and anaphase and now forms a slender arch. Its tips point toward the posterior border of the cell. In the anaphase they pointed toward the lateral boundaries.

From the point of its attachment to the nuclear membrane, a fibril is seen crossing the nuclear border of clear material and joining a rather broad eosin-stained line which traces the periphery of this border substance. This is the new axostyle. Like the mature form, it does not take Delafield's stain. It is to be noted further that from this clear border substance there are two unmistakable bands passing anteriorly, which seemingly connect also with the similarly clear core substance of the rostellar region.

If the length of the axostyle and the varying degrees of maturity of the nuclei are considered in figures 14, 15, and 16 of plate 66, a post-telophase anterior migration of the nucleus preceding cytoplasmic division must be presumed. Figure 15 represents an animal that is evidently much younger than the other two.

In this earliest stage of cytokinesis, the axostyles show a curiously angular crossing of about 90 degrees. The angle increases as division progresses (pl. 66, figs. 14, 16) until, in a very late stage (pl. 66, fig. 18), it measures 180 degrees. Figure 18 represents the condition when plasmotomy immediately follows nuclear division. However, if plasmotomy is delayed until the axostyles are longer and the nuclei have again attained the interkinetic state, the original 90-degree angle is lessened, and the axostyles appear as in plate 66, figure 19, with the distal tips apparently conjoined.

An anterior post-telophase migration would account for the identical increase in the number of nuclei and of axostyles usually seen in the multinucleate stages reported by Janicki (1915), Zelif (1930), and Connell (1930). Zelif reports a specimen of *O. minor* with four nuclei, but he also has a sketch which shows five. In this study, several specimens have been seen with two nuclei, one with three, and one with five. Only two of these showed more than one rostellum, and these were considered as representing early stages of plasmotomy. Casual observation indicates that in the multinucleate state, the combination of nucleus, axostyle, and blepharoplast remains essentially the same as in the uninucleates. On the basis of these observations, multinucleates may be explained as the result of anterior post-telophase migration and delayed plasmotomy.

The nucleus in the earlier stages of cytokinesis is uniformly filled with very coarse, dark granules. The dark chromatin mass next forms an anterior cap above a clear space within the nucleus, and the membrane becomes outlined with granules (pl. 66, fig. 14). Zelif (1930) speaks of the reappearance, in a central position, of the karyosome at the border of this chromatin cap. A similar stage has been seen in *O. minor*. It is not surmised, however, that the new karyosome forms from the remains of the centrodesmose (Zelif, 1930; Connell, 1930). This has disappeared much earlier.

In plate 66, figure 17, the karyosome is again in evidence and the dark chromatin cap has become diffuse. A slightly later stage is shown in the upper nucleus of figure 18, plate 66, in which the diffused chromatin fills the entire nucleus and the nuclear membrane appears as a solid dark line.

The lower nucleus in the same figure is accompanied by an axostyle that is much more mature than that of the cell just described. Evidently, reorganization within the two daughter cells is not necessarily synchronous. In the older nucleus, the chromatin is disposed in very small granules, an intermediate condition between the finely diffused organization preceding it, and the coarsely granular interkinetic nucleus following it.

Of the extranuclear organelles, the beginning of cytokinesis (pl. 66, fig. 15) shows only the immature axostyle, a granular blepharoplast, and possibly a rostellar fiber extending from the axostyle. With the upper nucleus of plate 66, figure 16, the blepharoplast seems to have divided and passed along the anterior border of the widened proximal end of the axostyle. With the lower nucleus these two granules have each divided to give the typical arrangement described for the interkinetic state.

SUMMARY

1. The percentage of *Oxymonas minor* in sections from the intestine of *Kalotermes minor* varies inversely with the size of the lumen and ranges from 30.6 to 85 per cent, which far exceeds the ratio obtained in smears.

2. The size and form of *O. minor* in sections and in smears have a wider range than has been previously reported, showing spheroids averaging 7.3μ in diameter and elongate animals averaging $24.7\mu \times 13.2\mu$.

3. The life cycle approximates that outlined by Connell (1930) for *O. dimorpha*. It comprises an attached and a motile phase. Rarely, the larger animals are found detached. The smaller animals appear in both the attached and the unattached state. Division is not confined to the larger animals, as Connell reported.

4. There is no evidence of formation of a centrodosome from the karyosome.

5. There is evidence of an anterior post-telophase migration of the nucleus preceding plasmotomy. This is of interest in considering the production of multinucleates from typically uninucleate animals by delayed cytokinesis. Further, if such a nuclear migration could be found preceding the transverse fission reported in a few Mastigophora, these would no longer appear as deviations from the pattern of reproduction in their class.

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PLATES

All drawings made with a camera lucida at a magnification of 3525, reduced one third (to $\times 2350$) in the reproductions. The sketches were made from smears except where it is stated that they were made from sections. Abbreviations: B, Bouin's solution; S, Shaudinn's fluid; F, Flemming's fluid; DE, Delafield's haematoxylin and eosin; H, Heidenhain's haematoxylin.

PLATE 64

Orymonas minor Hagen

Fig. 1. Intestinal intima retracted from the gut epithelium showing characteristic crowding of *Orymonas*. Section, B:DE.

Fig. 2. Interkinetic-type nucleus; arrowhead tip on axostyle. F:II.

Fig. 3. Prophase; early elongation of nucleus. S:II.

Fig. 4. Prophase; elongate nucleus with chains of chromatin. S:II.



PLATE 65

- Fig. 5. Prophase: diffused state of chromatin; centrodosome present. S:H.
- Fig. 6. Prophase: diffuse chromatin; karyosome broadened. S:H.
- Fig. 7. Prophase: chromatin largely diffuse; two karyosomes and centrodosome present. S:H.
- Fig. 8. Prophase; two karyosomes and granular reticulum. S:H.
- Fig. 9. Prophase; chromatin granules massing at equator between the two karyosomes. S:H.
- Fig. 10. Metaphase girdle; nucleus parallels long axis of body. Section, B:DE.
- Fig. 11. Early anaphase; nucleus tilting. S:H.
- Fig. 12. Late anaphase; nucleus tilted 90 degrees. F:H.
- Fig. 13. Telophase: reorganizing nuclei; new axostyles present. Section, B:DE.



PLATE 66

Fig. 14. Early plasmotomy: nuclei again in anterior position; regenerating organelles. F:H.

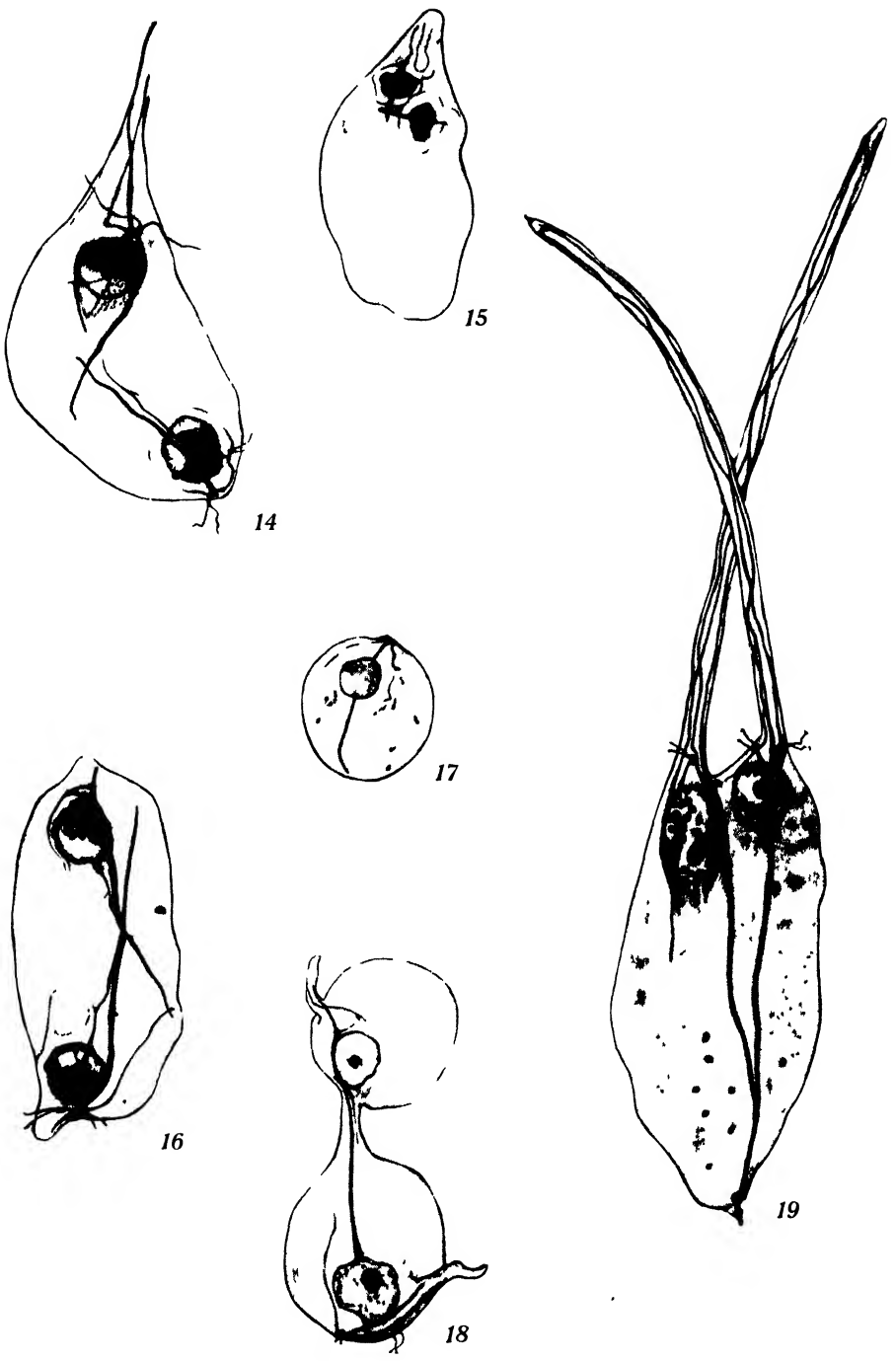
Fig. 15. An earlier stage in plasmotomy than the above. Notice the length of the axostyle and the immaturity of the nuclei. F:H.

Fig. 16. Later plasmotomy. Notice the length of the axostyles and their angular crossing. F:H.

Fig. 17. Reorganizing nucleus in daughter cell. Heavy chromatin cap becomes diffuse. Karyosome reappears. S:H.

Fig. 18. Very late plasmotomy. The axostyles drag across connecting cytoplasm. Unequal reorganization in the two cells. F:H.

Fig. 19. Delayed plasmotomy; two mature interkinetic nuclei. Notice the overlapping of the axostyle tips and compare with positions seen in figs. 14, 15, and 16.



PARTHENOGENESIS IN TERMITES OF THE GENUS ZOOTERMOPSIS

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BY
S. F. LIGHT

RAPID REPLACEMENT of lost reproductives of either sex is a characteristic feature of the colonies of the lower families of termites (Castle, 1934; Grassi and Sandias, 1893; Heath, 1903, 1927; Light, 1942-1943; Miller, 1942; Pickens, 1934) and of some genera of the higher termites (Bathellier, 1927; Emerson, 1933; Holmgren, 1906; Weyer, 1930). There was no reason at all, therefore, to expect to find parthenogenesis among termites. On the contrary, it seemed legitimate to assume that bisexual reproduction was obligatory; that the laying of eggs was itself an indication that insemination had occurred. This assumption could not readily be checked by observation, since copulation is rarely witnessed unless the groups or colonies are watched constantly.

Repeated experiments over a number of years have shown that this assumption is false. Unpigmented virgin female nymphs of *Zootermopsis angusticollis* and *Z. nevadensis* when isolated in groups are capable of becoming pigmented supplementaries and of laying numerous eggs entirely normal in appearance which hatch readily and give rise to seemingly normal young, as will be detailed below (see, also, Light, 1938; Light and McAuley, 1940).

As would be expected under the circumstances, the discovery of the capacity of the supplementary termite reproductive for parthenogenetic reproduction came about by chance. Heath (1927, 1928) had shown that certain soldier-like individuals of *Zootermopsis* were capable of carrying on reproduction. These we now know to be supplementaries determined as such so late in the soldier line that they possess many features of the true soldiers. At the time, the question arose whether these fertile soldiers were the result of some special development or whether all soldiers were capable of reproduction. To test the point a series of groups was set up from *Z. angusticollis* stock, each consisting of a male soldier with a number of unpigmented, supposedly virgin female nymphs. Some of the female nymphs of each of these groups became pigmented and eggs were laid which hatched into viable nymphs. Since at that time fertilization was thought to be a necessary precursor to development, these results were thought to prove that the male soldiers had functioned as males in these groups.

When the reciprocal mating was tested, however, female soldiers with male nymphs, no eggs were laid in any of the groups, although some of the male nymphs in each group became pigmented supplementaries. These results could be interpreted as an indication that whereas the male soldier was still able to function as a male, the female soldier had lost or had never attained reproductive maturity. Another possibility was, of course, that soldiers did not function as reproductives in either setup, but that the females reproduced parthenogenetically. To test the latter hypothesis a number of groups were isolated which consisted of unpigmented female nymphs only. Since in all these groups pigmented supplementaries developed and young were produced (Light, 1938; Light and McAuley, 1940), the conclusion was inescap-

able either that the nymphs of *Z. angusticollis* are capable of parthenogenetic reproduction or that many of the unpigmented, supposedly virgin nymphs are actually very precociously inseminated.

Since at this time only the fact of parthenogenesis was under consideration, the groups were used only to demonstrate that fact and to supply dated eggs for a projected study of the cytological mechanisms involved. No attempt was made to rear the young or to check on their sex. Later the young of two groups supposedly also made up of virgin females were found to consist of both males and females. This was, of course, a surprising situation, since parthenogenetically produced young are nearly always all males or all females, the latter condition being the more common and accompanied by a restoration of diploidy. The doubts raised by the unexpected finding were expressed when the fact of parthenogenesis was announced (Light and McAuley, 1940).

More recently a great many such groups of supposedly virgin females have given rise to young and many of these have been checked for sex characters and found without exception to be females. The female nymphs of these later groups, all of whose tested young were females, were carefully chosen from recently opened colonies, whereas those of the earlier experiments were taken from laboratory stock. It seems almost certain, therefore, that the two earlier groups whose young were tested and found to include some males included one or more females which, though not obviously pigmented, were already supplementary reproductives and had been inseminated previous to segregation. Since these early experiments were carried out we have found that, whereas all supplementaries ultimately become distinctly pigmented, the attainment of pigmentation may lag behind sexual functioning. Occasionally eggs are laid, for example, in groups of nymphs which contain no obviously pigmented individuals. Likewise, some unpigmented nymphs, or nymphs with pigment only on the seventh sternite, possess enlarged ovaries, large-yoked eggs, and functional development of the wall and lumen of the seminal receptacle and of the collateral glands. Such previously inseminated nymphs have been found, however, only in fragments of colonies headed by supplementaries, and not always in these. In colonies headed by primary pairs no supplementaries are found, and the reproductive organs of the female nymphs are immature. It is such nymphs, twice checked under the dissecting microscope for sex characters and absence of pigmentation, which have been used in our later experiments, to be reported here. In contrast with our earlier reports that the young produced, apparently by parthenogenesis, were of both sexes, in our current, carefully checked experiments all the young produced have proved to be females. These were all *Z. nevadensis*, but it seems practically certain that exactly the same conditions hold for *Z. angusticollis*. Even among the first series, two groups of which were found much later to contain offspring of both sexes, it seems probable that most of the groups produced female young only, and that it was only by an unusual chance that the two whose young were sampled included male offspring, presumably because some of the original females were already inseminated.

The first of the more recent series of groups of isolated female nymphs was set up in October, 1940, and designated 2PF. It consisted of 20 groups each composed of 20 female nymphs carefully checked with respect to sex and lack of pigmentation. These were taken from various colonies, but only from colonies freshly extracted. Some of the colonies of origin were, however, headed by supplementaries. As will be

seen, there is no evidence to show that any of the female nymphs chosen had been inseminated.

These groups were set up originally in order to obtain unfertilized eggs fixed at precise intervals after laying, with a view to making a study of the cytological

TABLE 1
NUMBERS OF YOUNG REMOVED AT RANDOM FROM GROUPS OF FEMALE
NYMPHS (SERIES 2PF AND 3PF) TO REPLACE DEATHS IN PFO

Date	Number	
	2PF	3PF
July 29, 1941	11	24
Aug. 5, 1941	4	10
Aug. 8, 1941 ..	0	2
Aug. 13, 1941 ..	4	10
Aug. 20, 1941	7	22
Aug. 27, 1941	6	12
Sept. 3, 1941	4	2
Sept. 10, 1941	3	3
Sept. 15, 1941	0	10
Sept. 24, 1941	5	0
Sept. 30, 1941	0	4
Oct. 3, 1941	3	0
Oct. 7, 1941	3	2
Oct. 30, 1941	0	28
Nov. 4, 1941	10	13
Nov. 12, 1941	0	10
Nov. 19, 1941	7	10
Nov. 26, 1941	4	13
Dec 2, 1941	4	14
Dec. 17, 1941	3	15
Dec. 30, 1941	6	15
Jan. 7, 1942	2	22
Jan. 21, 1942	1	19
Feb. 2, 1942	0	13
Feb. 17, 1942	0	10
Mar. 3, 1942	0	6
Apr. 3, 1942	0	9
June 5, 1942	0	5
Total females	87	303
Total males	0	0

mechanism involved in parthenogenesis in termites—information which technical difficulties have so far prevented us from obtaining. Early in 1941, hundreds of eggs were removed from series 2PF for cytological study, after which the groups were allowed to develop undisturbed.

From late June, 1941, to January, 1942, numerous young nymphs of the third, fourth, and fifth instars were removed at various times to form part of a series of groups composed of parthenogenetically produced individuals, and designated series PFO. All nymphs so transferred were carefully studied under the microscope and

found to be female. (The sex of nymphs cannot readily be determined earlier than the third instar.)

A second series of 30 groups of 20 female nymphs each, designated 3PF, was set up in January, 1941. All were from a single colony headed by a primary king and queen and containing no supplementaries. From late June, 1941, to late July, 1941, 110 nymphs of the third instar or later were removed from series 2PF and 3PF and combined in groups of 20 as PFO 1-5. All nymphs thus transferred were examined carefully and found to be female.

After these groups were set up, additional young were taken from 2PF and 3PF, as indicated in table 1, to replace losses by death in PFO 1-5 and to set up new groups in series PFO. The group origins of these nymphs were not recorded, but all groups of the series contributed young, and all were female. Since the 500 offspring of these 50 groups of isolated female nymphs chosen at random from all groups were all females, it seems very probable that no male nymphs were produced and, therefore, that parthenogenetically produced termites are females. Clearly, also, all the nymphs of the original groups (2PF and 3PF) which became supplementaries were virgin, which adds to the evidence that nymphs carefully selected for lack of pigmentation from colonies headed by primary reproductives, or even from those headed by supplementaries if from recently extracted colonies, are almost always virgin, if not always so.

Second-generation parthenogenetics.—As has been brought out, therefore, PFO was a series of groups of parthenogenetically produced female nymphs which had never been in the presence of males. PFO-1 with 20 such nymphs and PFO-2 with 15 were set up on June 26, 1941. Others were set up later. All these groups suffered high mortality; whether because of their parthenogenetic origin or because they were segregated while too young to care for themselves, was not determined.

The first egg was noted, in PFO-1, on September 9, 1941, some 56 days after isolation of the group. The first hatched nymph was seen much later, on March 24, 1942, and another on May 15, 1942. Others were noted, but in general the eggs were few, many were abnormally small, very few of them were seen to hatch, and all the young died before reaching the third instar. Hence no check for sex characters was made. Since, however, the young were certainly parthenogenetically produced by females which had never been in the presence of males, and since their mothers were from an exclusively female brood of supposedly virgin females, there seems every reason to believe that the second-generation parthenogenetics were also all females.

Whether parthenogenetic reproduction or the immaturity of the reproductive individuals explains the small number of eggs laid, their low hatching rate, and the low viability of the young and of their parents, remains to be determined.

In future experiments it is planned to use only very large female nymphs of very late instars, the so-called broad-headed nymphs (Light, 1942-1943), for the original groups. Young of these females can then still be distinguished by relative size even after they have reached the sixth instar, and groups of them should be, when segregated, as vigorous as groups of ordinary nymphs unless there is weakness arising from their parthenogenetic origin. It is hoped either to demonstrate the deleterious effects of continued parthenogenesis or to build up a large population of these parthenogenetically produced females in order to determine their capacity to continue to reproduce as an exclusively female line, and to develop into the various types found in the colony, particularly the alate type.

Parthenogenesis by primary females.—The experiments so far presented have involved only supplementary reproductives, or neotenics. When we were satisfied that parthenogenesis is regularly resorted to by the supplementary females which develop in groups of isolated females, it became of interest to determine whether the primary females derived by dealation from perfect females (alates) also possess the capacity for parthenogenetic reproduction. Consequently, numerous series were set up, some of which consisted of single female alates, others of two female alates, still others of trios. Here, as in the experiments with isolated female nymphs, the original purpose was to determine the occurrence of parthenogenesis. The determination of the sex of the offspring was an afterthought. Thus, the data are by no means so numerous as they might have been; but they are nevertheless convincing. Table 2 gives the essential information. Out of some 167 or more groups of primaries isolated as single alates, couples, or trios, 94 laid eggs, 74 were known to have had young, and 12 of these 74 were known to have produced the nanitic soldier characteristic of the incipient colony of termites (Light, 1942–1943). Altogether, 101 of the young produced by primary females which had been isolated as alates and therefore as virgins were examined for sex. Of the 101, 98 proved to be normal females. Three were anomalies, possibly bilateral gynandromorphs, since the posterior borders of the posterior sternites were convex on one side and concave on the other. None was clearly male.

One male was found in jar 3 of N2 females on September 22. At this time both primaries were dead and badly deteriorated, but a careful recheck on their sex, after restorative measures were taken, has shown that one was a male mistakenly introduced when the group was first constituted.

Only one experiment affords examples of possibly parthenogenetically produced males. A series of 42 groups was set up, each consisting of 1 female alate and 3 pale, unpigmented, presumably virgin, wing-padded nymphs. When the young of this series were segregated and their sex determined, 2 sixth-instar nymphs out of the total of 28 were males! A recheck of the surviving primaries and wing-padded nymphs showed them all to be females. Since the young were thrown together before their sex was determined, it is not known whether the two male nymphs came from the same group or from two groups. Several explanations present themselves to account for the appearance of a small proportion of males among the offspring of these groups of supposedly virgin females. Listed in what seems to be the order of increasing probability these are:

1. That there are parthenogenetically produced males.
2. That bisexually produced eggs were accidentally introduced into one or two of the groups and gave rise to the two males.
3. That the alate of one or two of the groups was by mistake a male instead of the female supposedly used.
4. That one of the wing-padded nymphs was by mistake a male and became a supplementary.
5. That one of the wing-padded nymphs was already inseminated, a supplementary not yet recognizable as such by its pigmentation.

The last seems by far the most probable explanation. One or more of the wing-padded female nymphs in each group became pigmented. These undoubtedly laid eggs. If one was inseminated, approximately half its offspring would be males.

TABLE 2
PARTHENOGENESIS AMONG PRIMARY FEMALES OF ZOOTHEROPSIS NEVADENSIS
(Alates were set up in late 1941 as singles, couples, or trios.)

Date set up	Designation	Number of groups	Constitution of groups	Number of groups laying	Number of groups with young	Number of groups producing soldier	Number of young determined as females	Number of young found to be anomalies
1941:								
Oct. 14-Nov. 5.....	NP ♀ ♀	28+	Couples of alates	28	21	7	7 soldiers 46 nymphs	0
Nov. 6.....	NT ♀ ♀	40	Couples of alates	21	18	1	1 soldier 22 nymphs	2
Oct. 14.....	NR ♀ ♀	16+	Singles for 5 weeks; then as couples	16	16	3	3 soldiers 13 nymphs	1
Dec. 23.....	N1 ♀	40	Singles	12	8	1	1 soldier 1 nymph	
1942:								
Feb. 18.....	N2 ♀ ♀	3	Couples from singles	2	1	0	0	0
1941:								
Dec. 23.....	N2 ♀ ♀	20	Couples	6	4	0	3	0
Dec. 23.....	N3 ♀ ♀	20	Trios, reduced in most groups to to couples or sin- gles	9	6	0	1	0
Total.....		167+		94	74	12	98	3

SUMMARY

1. Some nymphs in all groups composed of unpigmented female nymphs of *Zootermopsis* became pigmented and laid viable eggs.

2. The young of such females were females whenever the nymphs were from colonies headed by primaries and lacking in supplementaries, or even when the nymphs were derived from colonies headed by supplementaries, if these colonies had been recently extracted.

3. Primary females segregated as alates, and therefore virgin whether kept singly, in couples, or in trios, likewise gave rise to viable eggs. The young produced were all females, except for a few anomalous individuals no one of which was clearly male.

4. Groups consisting of single female alates isolated with several supposedly virgin female wing-padded nymphs gave rise to viable young. Two out of 28 such young were males; the remainder, females.

5. The males previously reported as occurring among the offspring of groups of isolated female nymphs (Light and McAuley, 1940), together with the two males occurring in groups of female primaries and wing-padded nymphs, may possibly have been parthenogenetically produced males. But it seems much more probable that these were bisexually produced, perhaps by precociously inseminated females or from eggs introduced by mistake during handling, or because a male was introduced by mistake.

6. Female termites, then, seem all to be capable of thelytokous parthenogenesis, the offspring thus produced being females, presumably diploid in chromosomal constitution.

7. Parthenogenetically produced females which had never been in contact with males produced viable eggs. The sex of these offspring was not determined, since they died in early instars, but it is presumed that they were females.

8. There is no evidence that the ability to reproduce parthenogenetically might be of significance in the life of the species. No completely or preponderantly female colony has been encountered, and functional males are present at all times in the colony, except, for a short period during which supplementary males are developing, in those relatively few colonies in which the founding male has died.

9. In the Isoptera, then, as seems to be true in several orders of insects, especially the orthopteroid groups, thelytokous parthenogenesis takes place readily when females are kept isolated from males, but seems not to occur, or to occur very rarely, under natural conditions and seems not to have arisen by selection nor to have any adaptive significance, but to be, in some way not understood, a chance outcome of the normal cytological processes of the animals of these groups.

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**EXPERIMENTAL STUDIES
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THE DEVELOPMENT OF SUPPLEMENTARY
REPRODUCTIVES IN THE TERMITE GENUS
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**BY
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EXPERIMENTAL STUDIES ON ECTOHORMONAL CONTROL OF THE DEVELOPMENT OF SUPPLEMENTARY REPRODUCTIVES IN THE TERMITE GENUS ZOOTERMOPSIS [FORMERLY TERMOPSIS]

BY
S. F. LIGHT

THE OCCURRENCE of neotenic reproductives, commonly called supplementary reproductives or supplementaries, in some of the colonies of most if not all species of termites is a well-established fact (see Light, 1942-1943). Fritz Müller, whose studies (1873) first emphasized the significance of supplementary reproductives in the termite colony, believed that they had superseded altogether the primary reproductive in the reproductive economy of the colony. Grassi (Grassi and Sandias, 1893) established the true role of the supplementaries in the colonies of *Kaloterms flavicollis* Fabr. and *Reticulitermes lucifugus* (Rossi), although perhaps exaggerating their importance in the latter species.

Grassi believed that the precocious sexual development of a few nymphs into supplementary reproductives, which occurs in each orphaned colony and in isolated groups of nymphs, results from special feeding of these particular nymphs by other members of the group. Heath (1931), although unable to establish the existence of a special diet, was inclined to accept the theory of special feeding as the most plausible explanation of caste determination in general. Thompson (1922) held that supplementaries represent separate castes in the colony and that the individuals of these castes are genetically determined from the egg and marked by different morphological features from the first instar. Snyder, who has contributed much of value to our knowledge of the termite castes and the constitution of the termite colony, accepted Thompson's theory of separate, genetically distinct castes of second-form (brachypterous) and third-form (apterous) reproductives, and has sought to prove it experimentally (Snyder, 1926, and Snyder and Popenoe, 1932).

The several studies of the termite colony growing out of the activities of the Termite Investigations Committee (Kofoid, Light, *et al.*, 1934), especially those of Pickens (1934, thesis MS, and 1943) on *Reticulitermes hesperus* Banks, those of Castle (1934*a*, *b*) on *Zootermopsis angusticollis* and *Z. nevadensis*, and my own somewhat more recent studies on the last-named two species, recently reviewed (1942-1943), as also the recent work of Miller (1942) on *Protrichotermes*, give no basis for belief either in an intrinsic determination of supplementaries as postulated by Thompson and by Snyder, or in their determination by special feeding as maintained by Grassi and presumably favored by Heath. On the contrary, all recent work supports the position strongly presented by Castle that all termite nymphs beyond the third instar possess an inherent tendency toward neoteny and that, given the proper conditions, any such nymph will develop into a supplementary reproductive. The proper condition for the development of nymphs into supplementaries has been found to be freedom from the inhibiting influences of functional reproductives.

Pickens (1932) formulated the inhibition theory, or, to use a more recent term, the

ectohormonal theory, and has recently reaffirmed it (1943). He postulates a chemical substance produced by the functional reproductives and spread throughout the colony by communal relations such as grooming—a chemical substance the effect of which on the recipient is to inhibit the action of the other mechanism, which latter presumably involves the action of a hormone or hormones and, in the absence of the inhibiting mechanism, would bring the nymph to precocious sexual maturity.

Castle accepted the ectohormonal theory as best explaining his results with *Zootermopsis* and presented (1934a) evidence that the inhibition is sex-limited, and that the absence of a female reproductive results in the appearance of female supplementaries only, whereas on the loss of the male only male supplementaries occur. This is not in agreement with the earlier findings of Grassi (1893) for *Kaloterme flavicollis*, nor is it in accord with my findings for *Z. nevadensis* (1942–1943), which indicate that the loss of either member of the founding pair results in the production of supplementaries of both sexes, although there is a preponderance of those of the absent sex and a tendency toward early elimination of those of the other sex. Further, it should be remembered that this type of inhibition, which I have termed contact inhibition, is complete only where it has continued undisturbed from the founding of the colony (Light, 1942–1943).

Castle (1934a, and thesis MS) presented some preliminary experiments which seemed to indicate that alcohol and ether extracts of female supplementaries when fed to groups of nymphs caused significant delay in the development of functional supplementaries, as indicated by a delay in egg laying. He performed three such experiments, each with groups of 50 nymphs of the fifth and sixth instars. In the first experiment, as in the last, all animals were from a single colony. The origin of the nymphs used in the second experiment is not stated, but it is presumed that these also came from a single colony.

The groups of one control series were fed on filter paper alone, and those of the others on filter paper treated with the various extracting media. In the first experiment there were three series of experimentals. One series of two groups was fed filter paper treated with extracts of functioning female supplementaries made in 70 per cent ethyl alcohol; another series of two groups was fed extracts of such supplementaries made with Locke's solution; and another series of two received extracts made with ether from the residue after extraction by means of alcohol. Egg laying in all groups in the first experiment except those fed alcohol extracts occurred at approximately the same time as in the controls. Castle assumed that the delay in the groups fed alcohol extracts indicated the presence of an inhibiting substance soluble in 70 per cent ethyl alcohol and that the failure of retardation in groups fed extracts made in Locke's solution indicated that this substance was not soluble in water. That the ether extract was ineffective he explained as due, presumably, to exhaustion of the substance during the previous extraction in alcohol. For this reason, in the third experiment one series of experimental groups was fed ether extracts and another alcohol extracts, both made directly from female supplementaries.

Table 1 gives the pertinent results of experiments 1 and 3. The striking differences in general between the two experiments with respect to the time before egg laying begins is perhaps to be attributed in part to differences between colonies, but probably in the main to seasonal effects. Experiment 1 was begun in December, when the reproductive activity of the colony is normally very low, whereas experiment 3 was

begun in June. It will be seen that in the groups fed alcohol and ether extracts in experiments 1 and 3, eggs were first recorded about two weeks later than in the other groups of the corresponding experiments. Castle naturally considered these findings to indicate that inhibiting substances extractable in ether and 70 per cent ethyl alcohol but not in water are produced by female supplementaries, and he predicted that with more refined methods an extract of material capable of completely inhibiting production of supplementary reproductives in groups of isolated nymphs would be attained.

The results of the second experiment were reported in the Ph.D. thesis only and there merely by the statement that "supplementary reproductives developed in the

TABLE 1
CASTLE'S EXPERIMENTS ON EXTRACT INHIBITION

Materials with which filter paper fed to termites was treated	Experiment 1 Average available dosage, 0.0016 of extract from one supplementary per nymph per day		Experiment 3 Average available dosage, 0.0029 of extract from one supplementary per nymph per day	
	Number of groups	Days to eggs	Number of groups	Days to eggs
Not treated.....	2	58, 60	2	39, 40
Ether.....	2	62, 62	2	41, 43
Alcohol (70 per cent ethyl).....	2	60, 62	2	42, 45
Locke's fluid.....	2	60, 60
Locke's extract of females.....	2	60, 60
Ether extract of females.....	3	52, 60, 62
Ether extract of residue after extraction with alcohol.....	2	62, 62
Alcohol extract of females.....	2	76, 83	3	52, 55, 55

experimental groups, as well as in the control groups, within 56 days." The exact times are not reported for the different groups, but it is presumed that there was no significant difference in this regard between the experimental and control groups since Castle held that the results were not significant. For the same reason it is presumed that eggs were laid at approximately the same time in all groups, although no mention of the time is made. It is to be noted that Castle attributed the negative results of this experiment to the low dosage, the average available dose being 0.0008 of one female supplementary per nymph per day. Since it has proved difficult to get consistent experimental confirmation of Castle's positive results, it seems necessary to keep these rejected negative findings in mind.

With the inhibitory effects of extracts of female supplementaries apparently proved, it became of interest to determine whether males produced a similar substance which would tend to inhibit the development of male supplementaries. Ridder sought to test this by feeding extracts of pigmented male supplementaries to groups of nymphs. He ground up the supplementaries in a mortar and extracted with ether. His results (thesis MS) are summarized in table 2.

The differences reported by Ridder between series fed male extracts and those not so fed are very great, and all the more striking in that pigmented females appeared almost simultaneously in all groups, irrespective of whether the groups were fed

extracts of functional males or not, and about at the same time as the appearance of pigmented males in those groups which were not fed extracts of supplementaries, whereas the appearance of pigmented males was strikingly delayed in the groups fed extracts of male supplementaries.

Table 2 shows further that egg laying was supposedly greatly delayed in the groups fed extracts of male supplementaries. Ridder considered this delay in egg laying to be additional evidence of an inhibiting effect of the extracts on males since

TABLE 2
SUPPOSED EFFECTS OF FEEDING EXTRACTS OF MALE SUPPLEMENTARIES
(From Ridder, 1935)

Treatment	Number of groups	Days to pigmentation				Days to first egg	
		Males		Females			
		Range	Mean	Range	Mean	Range	Mean
Paper only.....	4	21-22	21.75	21-22	21.5	29-31	29.5
Ether-treated paper.....	12	20-29	23.7	19-29	22.0	29-40	34.4
Ether extract of male alates.	8	15-31	22.5	15-31	20.6	29-43 ^a	34.0 ^a
Ether extract of seventh-instar males	8	20-24	22.0	20-24	22.6	32-40	35.7
Ether extract of male supplementaries.....	10	55-96 ^b	74.0 ^c	15-43	23.8	64-102 ^d	87.8 ^e

^a Omitting one group which died out.

^b None in one.

^c For nine colonies.

^d None in three.

^e For seven colonies.

he supposed that eggs were laid only after insemination had taken place. Since then (1938, 1940, 1942-1943, 1944) I have fully established the ability of virgin females to reproduce effectively in the absence of males.

Ridder's experiments are being repeated. The results will be reported later; until then it seems best to consider his findings to be of doubtful validity.

RECENT EXPERIMENTS SIMILAR TO THOSE OF CASTLE

The next attempt to verify the inhibitory effect of extracts was made by me in 1937 and will be discussed in a subsequent part of this report. At this point it seems more logical to present several of my experiments which followed more closely the pattern of Castle's experiments. The first of these, experiment X (table 3), was carried out in the fall of 1941, using 20 groups, each of 50 nymphs of *Z. nevadensis* of the fifth and sixth instars, all from a colony which consisted of 3,244 nymphs and 100 alates, and 34 broad-headed supplementaries (apterous supplementaries of eighth or later instar). Each group of one series of 10 groups (XS) was given weekly a filter paper impregnated with the ether extract from one female supplementary, prepared as in Castle's experiments; each of 5 groups (series XP) was given a filter paper impregnated with the ether extract of one unpigmented seventh-instar nymph; and each of 5 groups (XC) was given a filter paper treated with ether. The average available dose was 0.0029 of the extract of one individual per nymph per day, which was considerably more than in Castle's first experiment, but about the same as in his third (see table 1). As table 3 shows, there are no indications of any inhibiting effect from the feeding of extracts of supplementaries. Eggs were produced in all groups of the experimen-

tal series (XS), and on the average they appeared at least as early as in the controls. The total number of supplementaries produced per group was higher than in the controls. The mortality records seem to afford a partial explanation of these results. Five of the ten groups of controls (XC and XP) had more than a 60 per cent mortality by the eighth week, whereas in only one of the ten experimental groups (XS) did the population decline this much. Two of the control groups which were low in viability produced no eggs, and in four of these five groups of low viability fewer than six supplementaries were recorded, whereas only one of the experimental groups

TABLE 3
ESSENTIAL RESULTS OF EXPERIMENT X

XC Filter paper treated with ether and dried				XS Filter paper treated with ether extracts of one female supplementary per group per week			
Group	Days to first egg	Population at 8th week	Total supplementaries produced	Group	Days to first egg	Population at 8th week	Total supplementaries produced
1	No eggs	4	4	1	42	28	8
2	42	16	7	2	35	36	11
3	42	37	8	3	42	2	9
4	35	37	9	4	42	29	10
5	42	13	5	5	28	39	7
XP Filter paper treated with ether extracts of one seventh-instar nymph per group per week				6	42	23	9
				7	35	32	7
				8	42	34	5
				9	35	30	10
				10	42	30	6
1	35	39	9				
2	49	14	6				
3	35	34	8				
4	No eggs	6	5				
5	42	30	5				

produced fewer than six supplementaries. If the groups with noticeably low viability are ignored we find that the results in experimentals and controls are nearly the same, with an average of about 38 days to first egg and the production of about 8 supplementaries per group. Here, then, the inhibitory effect of the extract, if any, was more than offset by the differentially favorable conditions in the experimental series.

My next experiment (LC) followed the pattern of Castle's experiment in the method of extraction, but differed first in that the groups consisted of 30 nymphs instead of 50, and secondly in that, instead of fifth- and sixth-instar nymphs only, the groups included nymphs from the third to the eighth instar ("broad-headed nymphs") in a definite ratio (3 of the third and fourth instars, 8 of the fifth, 6 of the sixth, 9 of the seventh, and 4 broad-headed nymphs of the eighth or later instars). All were from one relatively small colony of about 2,500 nymphs headed by a primary pair.

Each of the 14 groups of the control series (LC-C, table 4) was fed weekly a filter paper impregnated with ether extracts of three young nymphs. The experimental groups, in three series of 15 each (LC-1, LC-2, LC-3), were fed weekly a filter paper

TABLE 4

ESSENTIAL RESULTS OF EXPERIMENT LC, TESTING EFFICACY OF EXTRACTS OF FEMALE SUPPLEMENTARIES IN RETARDING OR REDUCING EGG LAYING AND IN PRODUCTION OF SUPPLEMENTARIES

LC-C, controls, fed ether extract of three third- or fourth-instar nymphs				LC-1, fed ether extract of female supplementaries			
Group	Days to eggs	Total supplementaries at 10th week	Population at 10th week	Group	Days to eggs	Total supplementaries at 10th week	Population at 10th week
1	35	0	27	1	No eggs	0	21
2	No eggs	1	24	2	No eggs	2	22
3	42	2	27	3	56	2	20
4	35	4	22	4	56	2	24
5	No eggs	1	20	5	No eggs	1	22
6	35	5	24	6	63	5	24
7	35	0	25	7	35	2	27
8	42	2	24	8	35	3	27
9	56	3	19	9	35	2	16
10	56	2	22	10	No eggs	0	18
11	No eggs	2	20	11	42	2	24
12	No eggs	0	25	12	42	1	18
13	42	2	27	13	No eggs	2	23
14	14	No eggs	0	21
15	No eggs	2	19	15	56	2	22

LC-2, fed ether extract of female supplementaries				LC-3, fed ether extract of female supplementaries			
1	35	2	26	1	35	2	25
2	No eggs	2	21	2	35	2	25
3	70	2	15	3	35	2	20
4	35	2	19	4	35	2	16
5	35	2	25	5	35	1	27
6	No eggs	3	22	6	No eggs	1	22
7	56	0	24	7	56	3	24
8	35	2	22	8	35	3	22
9	42	2	27	9	35	4	20
10	No eggs	1	18	10	49	3	22
11	63	3	22	11	42	2	25
12	No eggs	0	21	12	42	2	25
13	35	2	21	13	56	2	13
14	No eggs	2	20	14	35	4	14
15	35	2	24	15	35	2	27

impregnated with the ether extracts from one female supplementary, an average available dose of 0.0028 of the extract of one supplementary per nymph per day. Beginning with the fourth week, observations were made weekly with respect to numbers of eggs and supplementaries present. Table 4 gives essential detailed results. From tables 5 and 6 it will be seen at once that the three series of experiments, supposedly made up of groups of the same constitution and treated in exactly the same way, gave different results and that the results of one experimental series were higher than the results from the control series, and those of another were lower. One of the experimental series, LC-3, stood out as having early laying of eggs, pro-

duction of more supplementaries (tables 5 and 6), and laying of more eggs than the control. Further, 14 of its 15 groups produced eggs in the 9 weeks of the experiment, whereas in each of the other three series 5 to 6 groups were recorded as not producing eggs. Without this third series the first two experimental series might have been thought to show some inhibition. The differences between the two experimental

TABLE 5
FREQUENCY DISTRIBUTION OF GROUPS IN THE FOUR SERIES OF EXPERIMENT LC
BY NUMBERS OF DAYS TO FIRST EGG

Series	Days to first egg							Average days for groups producing eggs	Average if those without eggs are counted as with eggs at last observation
	35	42	49	56	63	70	No eggs		
LC-C.....	4	3	0	2			5	42 0	52.0
LC-1.....	3	2	0	3	1		6	46 67	56.0
LC-2.....	6	1	0	1	1	1	5	44 1	52.7
LC-3.....	9	2	1	2			1	40.0	42 0

TABLE 6
FREQUENCY DISTRIBUTION OF GROUPS IN THE FOUR SERIES OF EXPERIMENT LC
BY NUMBERS OF SUPPLEMENTARIES PRODUCED

Series	Numbers of supplementaries produced						Average number per group
	0	1	2	3	4	5	
LC-C.....	3	3	5	1	1	1	1 8
LC-1.....	3	2	8	1	0	1	1.7
LC-2.....	2	1	10	2	0	0	1.8
LC-3.....	0	2	8	3	2	0	2.3

series LC-1 and LC-3 are much greater than those between any experimental series and the controls and therefore can have no significant correlation with extract inhibition.

In connection with experiment LC it should be remembered that the presence of broad-headed nymphs makes this a very different situation from that of Castle's experiments, since, as we now know (Light, 1942-1943), broad-headed nymphs tend to become supplementaries very early, and it seems probable that only a strongly inhibiting influence would prevent them from doing so. Such differences as were recorded seem significant of differences in the health of the various series. This is not so clearly indicated by the mortality as by the numbers of groups which produced no eggs. Any such group under normal conditions would produce eggs in less than 9 weeks, yet 5 out of 14 in the control series, and 6 and 5, respectively, out of the 15 each in LC-1 and LC-2 failed to do so, whereas all but one did so in LC-3. Here, then, as in experiment X, we have no indication of an inhibitory effect from supplementary extracts, but a very good indication of the degree of the differences which are to be expected from the operation of unidentified fluctuating variables.

The third experiment of the type performed by Castle (EX) consisted of 60 groups, each of 50 sixth-instar nymphs of *Z. nevadensis*, set up in January, 1942. All

were from a single large colony consisting of more than 12,000 individuals headed by a number of supplementary reproductives. One series of controls (EX-N, table 7, b), consisting of 20 groups, was fed filter paper bearing ether extracts of unpigmented nymphs; another (EX-C, table 7, a), filter paper treated with ether. The experi-

TABLE 7, a
CONTROL SERIES, EX-C, OF EXPERIMENT EX

(The groups of this series were fed filter paper previously treated with ether and dried. Population and number of supplementaries are given for each group at approximately biweekly intervals beginning with the twenty-fifth day. The figures for supplementaries include those known to have died.)

Group No.	Days after setting up experiment									
	25		39		53		67		81	
	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.
1.....	49		49		48	4	44	6	40	7
2.....	48		45	1	45	5	40	8	37	9
3.....	47		46		44	3	44	4	40	4
4.....	48		49		46	2	44	7	42	7
5.....	48		43		42	4	41	4	40	4
6.....	48		44		43	4	43	6	39	7
7.....	48		47		47	3	46	5	42	5
8.....	49		44		43	2	41	3	41	9
9.....	50		46		46	2	46	3	44	6
10.....	49		47		47	1	46	2	44	6
11.....	48		47		47	3	47	4	46	4
12.....	49		46		43	3	39	4	39	5
13.....	48		45		45	4	43	7	39	7
14.....	46		41		38	4	36	4	36	5
15.....	49		49		48	3	47	4	43	7
16.....	45		45		44	3	42	5	41	6
17.....	50		49		48	6	47	7	46	9
18.....	47	2	45	2	45	6	44	7	42	8
19.....	48		44		44	3	43	3	39	6
20.....	48		45	4	44	4	42	6	42	8

mental series (EX-S, table 7, c) was fed filter papers treated with ether extracts of female supplementaries. The results given in tables 7 to 11 show that the series fed extract of supplementaries (EX-S) lagged in time of egg laying and in numbers of supplementaries developed. By the sixty-seventh day, for example, eggs had been produced (table 8) by all groups in the two control series, whereas three groups in EX-S were without eggs, and the difference in numbers of supplementaries produced, brought out in tables 8 and 10, is even more striking. In only one of the 20 groups in the experimental series was the number of supplementaries produced higher at the sixty-seventh day (tables 8 and 10, a) than the mean of either control series. Table 10, b, shows similar although somewhat less striking differences maintained as late as the fourteenth week. These results have greater validity than Castle's because of the much greater numbers of groups involved and because full information is available with respect to the incidence of mortality.

In comparison with my other experiment (X) modeled on Castle, and my variant of it (LC), the results of EX seem to be more valid (1) because the mortality was low throughout all series (see table 7), namely, less than 25 per cent for the 95 days of the experiment, (2) because no group died out during the experiment, and (3) because every group produced eggs. All three facts indicate approximately comparable

TABLE 7, b

CONTROL SERIES, EX-N, OF EXPERIMENT EX

(Groups fed filter paper impregnated with extracts of unpigmented nymphs. Figures for supplementaries include those known to have died.)

Group No.	Days after setting up experiment											
	25		39		53		67		81		95	
	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.
1.....	47		42		19	4	11	5	10	6	10	6
2.....	49		46	1	33	1	30	7	30	7	30	7
3.....	49		46		39		35		34	4	34	4
4.....	49		47		39	2	39	3	39	4	38	4
5.....	49		44		41	3	40	7	39	9	37	10
6.....	50		40		44		43	3	42	4	41	5
7.....	48		46		45		44	4	43	4	42	5
8.....	48		46		42	2	40	4	39	8	39	9
9.....	47		47		39	1	38	5	38	6	37	6
10.....	48		45		41		39	3	38	4	36	5
11.....	49		46		42	3	40	8	39	9	37	9
12.....	49		49	1	48	2	47	5	46	7	44	8
13.....	50		48		44	1	44	2	43	4	40	8
14.....	48		48		44	1	44	6	43	7	41	8
15.....	49		44		42	3	42	5	40	7	40	8
16.....	49		47		44		41	8	41	8	39	10
17.....	50		50		47	2	45	3	45	6	42	6
18.....	48		47	1	45	2	44	7	43	7	43	10
19.....	49		48		45	1	41	6	40	7	40	9
20.....	49		45		43	1	43	2	42	5	42	6

normal conditions in all series and in nearly all groups. Finally, as tables 7, 8, and 11 show, mortality was actually lower in the experimental group from the twenty-fifth day on than in either control group, and hence there is no evidence that the lag in reproductivity in the experimental series is to be accounted for by unfavorable conditions.

An application of statistical formulas to the results of Castle's first and third experiments and of experiment EX shows that the frequency distribution of groups by days elapsed to egg laying is significantly different as between the experimentals and those considered as controls. Since there is no other obvious factor to explain these differences, it seems necessary to attribute them tentatively to an inhibiting effect from extracts of supplementaries. Until much more striking inhibition is obtained, or until results similar to those of experiment EX can be produced consistently, or until the factors which result in high mortality correlated with very low reproductivity in experiments of this type can be controlled or evaluated, it cannot be said that inhibition by Castle's methods has been proved.

THE 1937 EXPERIMENT

In an experiment carried out in the spring of 1937, resort was had to supposedly more effective methods of extracting the inhibiting material, and heavier doses were administered. By these means it was hoped to produce a much more significant

TABLE 7, c
EXPERIMENTAL SERIES, EX-S, OF EXPERIMENT EX

(Groups fed filter paper impregnated with extracts of female supplementaries. Figures for supplementaries include those known to have died.)

Group No.	Days after setting up experiment									
	25		39		53		67		81	
	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.	Pop.	Sup.
1.....	48		44		42	2	37	2	33	4
2.....	50		49		48		46	1	45	4
3.....	49		46		44	1	38	2	35	4
4.....	49		47		45		45		35	4
5.....	50	1	49	1	49	4	46	4	42	6
6.....	48		47		47	2	45	3	41	7
7.....	49		48	1	46	2	41	3	39	3
8.....	47		42		40	1	40	1	38	4
9.....	50		48	1	48	4	44	4	43	6
10.....	49		48		48	1	46	2	46	2
11.....	49		47		42	1	40	3	37	5
12.....	50		49		44	2	38	2	37	6
13.....	50		47		45		45	3	45	5
14.....	49		46		46		45	1	45	5
15.....	48		46		45	2	43	4	43	7
16.....	50	1	49	1	47	2	47	4	45	6
17.....	50		50		47	3	43	5	41	7
18.....	48		47	1	47	1	45	4	44	4
19.....	50		49	1	49	2	48	3	48	5
20.....	50		48		47	1	46	2	46	3

delay in the onset of reproductivity, if not its complete inhibition. Further, it was hoped that some indication of the location of the organ or organs secreting the inhibiting substance might be obtained. Methods of extraction were developed with the aid of Dr. O. H. Emerson, and the experiment was under the immediate supervision of Dr. Olga Hartman. Results were presented in abstract form and published in the *Anatomical Record* (Light, Hartman, and Emerson, 1937). The detailed findings were never published, however, because of doubts raised by the complexity of the theory deemed necessary to explain the results, and because of uncertainty with respect to the validity of certain experimental procedures. Hence the results are presented here in detail for the first time.

The groups used in the experimental and control series consisted of 10 nymphs each, all of about the same instar, presumably the fifth or sixth since many of the nymphs developed wing pads shortly after the experiment was set up. Each group was housed in a one-ounce ointment jar with metal screw top. The nymphs were obtained from various colony fragments in the laboratory, presumably representing

several different colonies, chiefly of *Zootermopsis angusticollis*, but there is reason to believe that some were actually from laboratory groups of *Z. nevadensis*.

Twenty groups constituted the control series, and series of twenty groups were used for each of the six extract-feeding experiments. Thus, 1,200 individuals figured in the experiments, in addition to the 200 controls. All groups were fed on Whatman's Filter Paper No. 1, each group of ten receiving a circle 3 cm. in diameter each

TABLE 8
RESULTS IN EXPERIMENT EX SUMMARIZED BY SERIES

EX-C: Fed filter paper treated with ether												
	Days after starting											
	20	25	32	39	46	53	60	67	74	81	88	95
Groups with eggs	0	0	0	0	1	8	20	20	20	20	20	20
Supplementaries	1	2	2	7	16	69	70	99	104	127	136	143+
Mortality.....	30	38	51	87	98	103	117	135	157	178	188	202
EX-N: Fed ether extracts of unpigmented nymphs												
	Days after starting											
	20	25	32	39	46	53	60	67	74	81	88	95
Groups with eggs	0	0	0	0	0	9	17	20	20	20	20	20
Supplementaries	0	1	2	3	4	29	34	93	108	123	132	143
Mortality.....	13	26	52	70	129	174	195	210	220	226	240	248
EX-S: Fed ether extracts of female supplementaries												
	Days after starting											
	20	25	32	39	46	53	60	67	74	81	88	95
Groups with eggs	0	0	0	0	1	5	14	17	20	20	20	20
Supplementaries	1	3	3	6	16	31	35	53	93	97	108	115
Mortality.....	15	17	32	54	72	84	101	132	164	172	181	190

week. The preparations in solution were allowed to dry on the filter papers and reached the animals as the paper was eaten. The controls received untreated papers. Every third week a piece of wood somewhat altered by fungus was given to each group of all series to supply dietary deficiencies; it was allowed to remain for a week.

Thirty-three functional supplementary queens were used each week in preparing the extracts, a number dictated by the available stock. The body of each of these female supplementaries was divided into three parts, with a view to locating roughly at least the source of the effective inhibiting substance. The viscera, including the alimentary tract and reproductive organs, were segregated by seizing the extreme posterior end of the abdomen with fine forceps and pulling gently while the head was held down. The head, with the prothorax, was then separated from the abdominal portion of the body wall. The three portions were ground separately with water and the resulting materials dispersed in water, which was filtered after standing for some time. The residues were extracted with absolute methyl alcohol. The resulting six filtrates were concentrated by boiling to a volume of 5 cc. each, and 0.25 cc. of

TABLE 9

FREQUENCY DISTRIBUTION OF GROUPS IN THE SERIES OF EXPERIMENT EX BY DAYS TO FIRST EGG

Series	Days to first egg				
	46	53	60	67	74
EX-C.....	1	7	12	5	
EX-N.....	0	9	8	3	
EX-S.....	1	4	9	3	3

TABLE 10

FREQUENCY DISTRIBUTION OF GROUPS IN SERIES OF EXPERIMENT EX
BY NUMBERS OF SUPPLEMENTARIES PRODUCED

Series	A. At 67th day										Average number per group	
	Numbers of supplementaries											
	0	1	2	3	4	5	6	7	8			
EX-C.....	0	0	1	3	6	2	3	4	1		4.95	
EX-N.....	1	0	2	4	2	4	2	4	1		4.60	
EX-S.....	1	3	5	5	5	1	0	0	0		2.65	
Series	B. At 95th day											Average number per group
	Numbers of supplementaries											
	0	1	2	3	4	5	6	7	8	9	10	
EX-C.....	0	0	0	0	2	3	3	3	2	4	3	7.20
EX-N.....	0	0	0	0	2	3	4	1	4	3	3	7.15
EX-S.....	0	0	1	2	4	4	1	2	3	3	0	5.75

TABLE 11

SUMMARY OF RESULTS IN EXPERIMENT EX

Series	Average number of days to first egg	Average number of supplementaries per group at 95th day	Average population at 53d day
EX-C.....	56.85	7.20	44.8
EX-N.....	57.90	7.15	41.3
EX-S.....	61.00	5.75	45.8

one of the concentrated extracts was used to impregnate the filter paper fed to a single group. Thus each group received weekly a particular extract (water or methanol) from a particular part of the body (head-thorax, or viscera, or abdominal wall) derived from approximately 1.65 female supplementary reproductives. Theoretically, each individual received one-tenth of this amount, an average available dose of 0.0235 of the extract from the particular part of one female supplementary per nymph per day. It is safe to say, however, that in all such experiments the actual dose averages considerably less than the theoretical dose since the treated paper is never completely consumed.

Observations were made weekly from the third to the thirteenth week after the first feeding. Table 12, A-G, gives the complete results. Records were kept of the numbers alive in each group, the presence or absence of eggs, the numbers of pigmented individuals present and the degree of their pigmentation (measured in three degrees: p^1 = slightly pigmented; p^2 = somewhat pigmented; p^3 = definitely pigmented). The criterion of reproductivity used was that employed by Castle, that is, days to laying of first egg. Record was kept also of the pigmented individuals, but since it had been planned to use egg laying as the criterion, each group was removed from consideration as soon as it was found to have eggs. This has made it very difficult to get a valid picture of the trends in the different series with respect to mor-

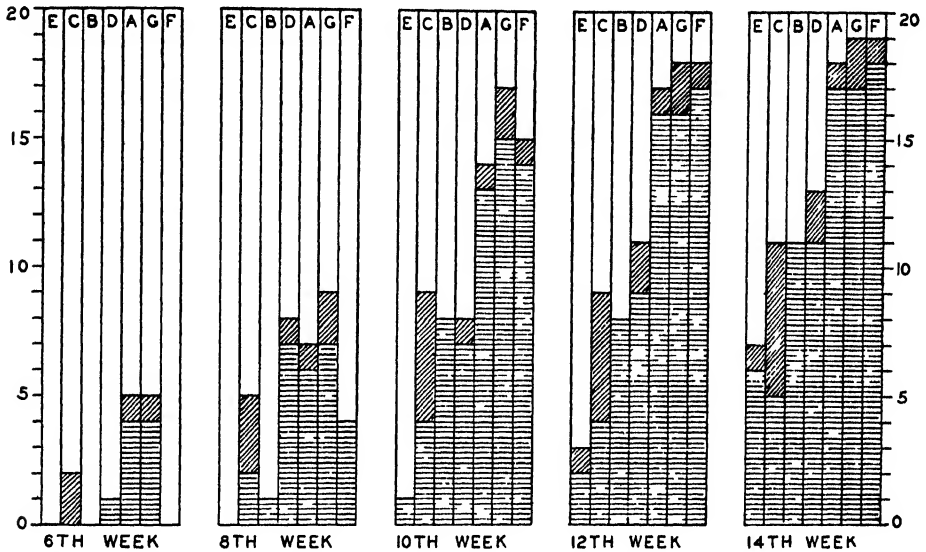


Fig. 1. Bar diagrams showing for the seven series of the 1937 experiment from the sixth to the fourteenth week (1) numbers of groups in which eggs had been laid (horizontal hatching), (2) numbers of groups which had died out (oblique hatching), and (3) numbers of extant groups for which eggs had not been recorded (blank). The series are arranged in the order of over-all increase in reproductivity, supposedly the order of decreasing inhibition.

A, controls; B, series fed methanol (100 per cent methyl alcohol) extract of head-thorax of supplementary reproductives; C, series fed methanol extract of viscera; D, series fed water extract of abdominal wall; E, series fed water extract of head-thorax; F, series fed water extract of viscera; G, series fed methanol extract of abdominal wall.

tality and development of supplementaries for the latter period of the experiment. There arises also the question of those groups which died out while the experiment was in progress. As figure 1 shows, in one series (B), which was fed alcohol extracts of the head-thorax, no groups died out, whereas five of the other six series lost one or two groups each, and one (C), fed alcohol extract of the viscera, lost six groups. One may omit from consideration groups which died out during the experiment. This unfairly weights those series which include groups of high mortality which did not die out. On the other hand, if the groups which died out are counted in, the result is to weight unduly the mortality and to dilute the reproductivity of the series (C) which had six such groups.

[illegible]

8th week—IX-8-37: Population Supplementaries: p ¹ p ² p ³ Eggs	10	10	8	9	10	10	10	10	10	8	9	9	9	8	2	8	9	8	8
	1		1	9	10	2	9	10	10		1	2	9				4	1	1
				9	10		9	10	10		3	10	10						
				9	10		9	10	10		3	10	10						
				9	10		9	10	10		3	10	10						
9th week—IX-14-37: Population Supplementaries: p ¹ p ² p ³ Eggs	10	10	8	9	10	10	10	10	10	8	9	8	9	8	8	8	9	8	8
			1	9	10		9	10	10				9				4	1	1
			1	9	10		9	10	10				9						
			1	9	10		9	10	10				9						
			1	9	10		9	10	10				9						
10th week—IX-22-37: Population Supplementaries: p ¹ p ² p ³ Eggs	9	10	8	9	9	9	9	9	9	8	8	8	9	8	8	8	9	8	8
			1	9	9		9	9	9				9				4	1	1
			1	9	9		9	9	9				9						
			1	9	9		9	9	9				9						
			1	9	9		9	9	9				9						
11th week—IX-30-37: Population Supplementaries: p ¹ p ² p ³ Eggs	9	9	7	7	8	8	7	8	8	8	8	8	9	8	8	8	9	8	8
			1	7	8		7	8	8				9				4	1	1
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						
12th week—X-7-37: Population Supplementaries: p ¹ p ² p ³ Eggs	9	9	7	7	8	8	7	8	8	8	8	8	9	8	8	8	9	8	8
			1	7	8		7	8	8				9				4	1	1
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						
13th week—X-14-37: Population Supplementaries: p ¹ p ² p ³ Eggs	7	1	7	7	8	8	7	8	8	8	8	8	9	8	8	8	9	8	8
			1	7	8		7	8	8				9				4	1	1
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						
			1	7	8		7	8	8				9						

8th week—IX-8-37 Population Supplementaries p ¹ p ² p ³ Eggs	2	2	0	8	9	4	7	9	7	0	10	8	2	9	10	0	9	4	9	6
	1			1	1	1	1	1	2	0					1	0	2			
			0					+							+					
9th week—IX-14-37 Population Supplementaries p ¹ p ² p ³ Eggs	2	2	0	8	9	4	7	9	2	0	10	8	1	8	10	0	9	4	9	6
	1			1	1	1	1	1	1			1		1		0	2			
			0				1	+						3						
														3						
10th week—IX-22-37 Population Supplementaries p ¹ p ² p ³ Eggs	2	0	0	8	9	4	7	9	2	0	10	7	0	8	10	0	9	3	8	6
	1	1		1	1	1	1	1	1		1	1	1	1		0	2		1	
			0			2	1	+						4		0	+			
														+						
11th week—IX-30-37 Population Supplementaries p ¹ p ² p ³ Eggs	1	0	0	8	9	4	7	9	2	0	7	6	0	1	10	0		3	7	5
	1	1		1	1	1	1	1	1		1	1	1	4		2	2			
			0				1	+					0	+		0	+			
12th week—X-7-37 Population Supplementaries p ¹ p ² p ³ Eggs	1	0	0	8	9	4	7	9	2	0	6	6	0		10	0		3	7	5
	1	1		1	1	2	1	1	1		1	2	1	1	1	2	2	1	1	
			0				1	+				1	0	+		0	+			
												1	0							
												1								
13th week—X-14-37 Population Supplementaries p ¹ p ² p ³ Eggs	0	0	0	8	9	4	7	9	2	0	6	6	0		10	0		3	7	5
	1	1		1	1	2	1	1	1		1	2	1	1	2		2	1	1	
			0				1	+				1	0	+		0	+			
												1								
												1								

TABLE 12—(Continued)
E. Series E, fed water extract of head-thorax

	Group no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3d week—VIII-11-37: Population..... Supplementaries: p ¹ p ² p ³	3	10	9	10	9	10	10	10	10	10	10	10	10	10	10	9	10	10	10	9

4th week—VIII-18-37: Population..... Supplementaries: p ¹ p ² p ³	3	10	9	10	9	10	9	10	10	10	10	10	9	10	10	9	10	10	10	8
	1
	1

5th week—VIII-25-37: Population..... Supplementaries: p ¹ p ² p ³	2	10	9	10	9	10	9	10	10	10	10	10	9	10	8	9	10	9	10	8
	1
	1	1	1

6th week—IX-2-37: Population..... Supplementaries: p ¹ p ² p ³	2	10	9	10	9	10	9	10	10	10	9	10	9	10	8	9	10	9	9	8
	1
	3	..	2	1	1

7th week—IX-8-37: Population..... Supplementaries: p ¹ p ² p ³	2	10	9	10	9	10	8	10	10	10	9	10	9	10	8	9	10	9	9	8
	1

	1	..	3	..	3	1	..	1	1

8th week—IX-14-37: Population Supplementaries: p ¹ p ² p ³ Eggs	7	9	9	8	8	9	10	10	10	3	1	1	10	10	8	10	10	10
9th week—IX-22-37: Population Supplementaries: p ¹ p ² p ³ Eggs	4	9	9	8	7	9	10	10	10	3	3	3	9	10	0	9	10	10
10th week—IX-30-37: Population Supplementaries: p ¹ p ² p ³ Eggs	4	9	9	8	8	9	10	10	10	3	3	3	9	10	0	9	10	10
11th week—X-7-37: Population Supplementaries: p ¹ p ² p ³ Eggs	4	9	9	8	8	9	10	10	10	3	3	3	9	10	0	9	10	10
12th week—X-14-37: Population Supplementaries: p ¹ p ² p ³ Eggs	4	9	9	8	8	9	10	10	10	3	3	3	9	10	0	9	10	10
13th week—X-21-37: Population Supplementaries: p ¹ p ² p ³ Eggs	4	9	9	8	8	9	10	10	10	3	3	3	9	10	0	9	10	10

Certain features of these results are obvious, however they may be formulated. First, not only the controls (A) but also two other series (F, G) show relatively early and complete development of supplementaries and laying of eggs (figs. 1-3; tables 12, 13). By the fourteenth week all living groups of controls but two had laid eggs (A, fig. 1), and all but one of series G, fed alcohol extract of abdominal wall, and of series F, fed water extract of the viscera. Likewise, in terms of supplementaries, however weighted, these three series were much more active than the others

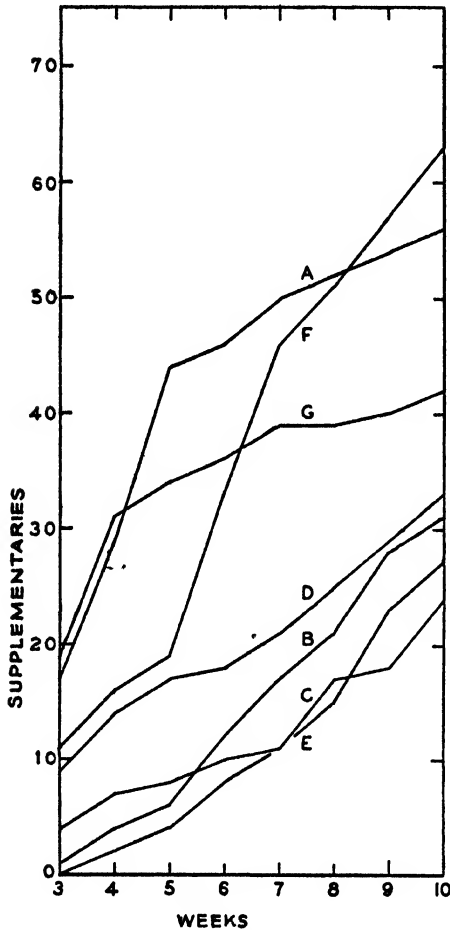


Fig 2

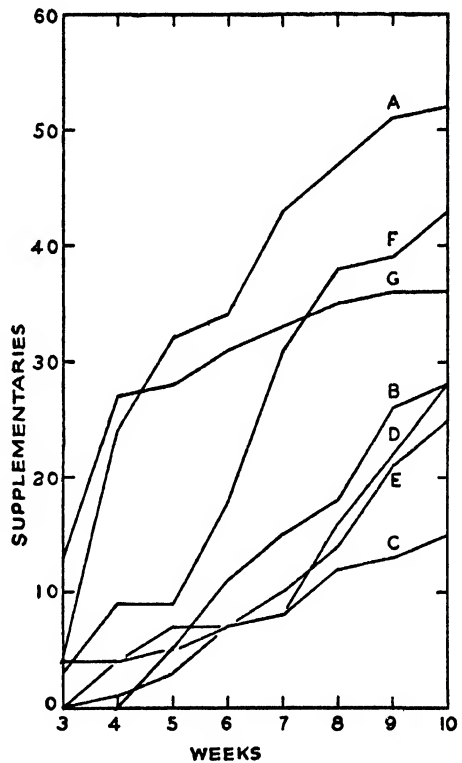


Fig. 3

Fig. 2. Frequency polygons showing weekly totals of pigmented supplementaries for the seven series of the 1937 experiment for the third to tenth weeks. Supplementaries known to have died are included in the totals, as are those of groups discarded because of egg laying.

A, control; B, fed methanol extract of head-thorax; C, fed methanol extract of viscera; D, fed water extract of abdominal wall; E, fed water extract of head-thorax; F, fed water extract of viscera; G, fed methanol extract of abdominal wall.

Fig. 3. Frequency polygons for the 1937 experiment showing weekly totals of somewhat pigmented and distinctly pigmented supplementaries (omitting slightly pigmented individuals). Totals continued for groups discarded because of death or egg laying and for supplementaries known to have died in extant groups.

(table 13, figs. 2 and 3). It should be kept in mind that by the tenth week so many groups had been discarded from among the uninhibited series (A, F, and G) as having laid eggs that comparisons based on production of supplementaries cease to have validity, and that other distortions arise from differences between series with respect to dying out of groups.

TABLE 13

FREQUENCY DISTRIBUTION OF GROUPS IN THE SEVEN SERIES OF THE 1937 EXPERIMENT BY NUMBERS OF SUPPLEMENTARIES PRODUCED BY THE EIGHTH WEEK
(Supplementaries known to have died are counted in)

Series	Numbers of supplementaries						Average number per existing group
	0	1	2	3	4	5	
E	9	9	0	2	0	0	0 75
C	11	5	1	2	1	0	0 85
B	8	6	4	1	1	0	1 05
D	7	4	7	1	1	0	1 25
A	0	1	10	5	4	0	2 60
G	2	3	10	4	1	0	1 95
F	1	4	6	3	3	3	2 60

TABLE 14

PRELIMINARY CONCLUSIONS FOR THE 1937 EXPERIMENT IF DIFFERENCES IN REPRODUCTIVITY ARE TO BE INTERPRETED SOLELY IN TERMS OF INHIBITION DUE TO FEEDING OF EXTRACTS

Part extracted	Effects of water extract	Effect of methanol extract of residues from water extraction
Head-thorax	Marked inhibition (E)	Seeming inhibition (B)
Abdominal wall	Seeming inhibition (D)	No inhibition (G)
Viscera	No inhibition (F)	Seeming inhibition (C)

In striking contrast to these three series (A, F, and G), which apparently were uninhibited, was the behavior of the series fed water extract of head and thorax (E). This series produced no eggs until the eleventh week (fig. 1), and only two of its groups had produced eggs by the end of the thirteenth week; the number of supplementaries produced was very low (fig. 2). In each of the remaining three series (C, B, and D), which were fed, respectively, methanol extract of viscera, alcohol extract of head-thorax, and water extract of abdominal wall, a considerable number of groups failed to produce eggs by the fourteenth week (fig. 1) and the number of supplementaries produced by them was markedly lower than in A, F, and G (figs. 2 and 3). It was naturally concluded that certain regions of functioning reproductives contained a substance or substances which when extracted and fed to isolated groups of nymphs resulted in a limited inhibition or retardation of reproductivity (Light, Hartman, and Emerson, 1937).

Table 14 summarizes the general results of the 1937 experiment if they are interpreted as due to inhibitory effects of extracts. The obvious explanation would postulate two inhibiting substances, the first soluble in water and methanol and present in greatest abundance in the head-thorax and in smaller amount in the abdominal wall,

and a second substance in the viscera soluble in methanol but not in water. The diminished effect of the methanol extract of head-thorax would be explained on the basis of low concentration after water extraction; or a different, methanol-soluble substance might logically be postulated. The lower effectiveness of water extract of abdominal wall as compared to that from water extracts of the head-thorax would presumably indicate that smaller amounts of the material were to be found there.

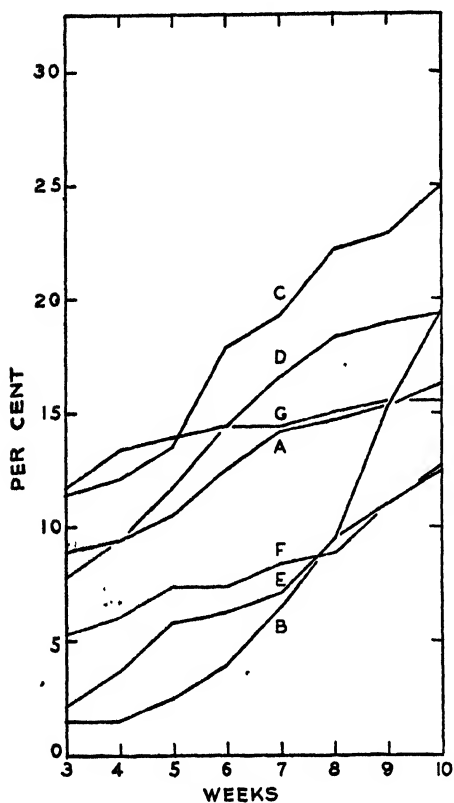


Fig. 4

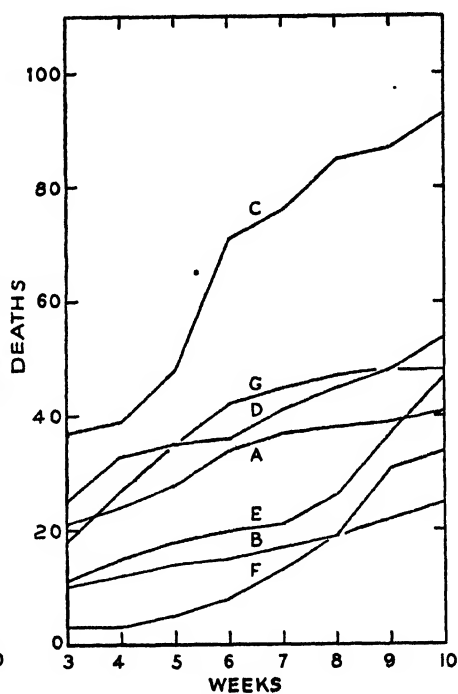


Fig. 5

Fig. 4. Frequency polygons showing mortality in terms of per cent of the original population of those groups of the seven series of the 1937 experiment which survived the experiment. Mortality of groups which were discontinued because of egg laying is continued as of the last week they were extant.

Fig. 5. Frequency polygons showing mortality in terms of total dead for all groups of the seven series of the 1937 experiment. Deaths in groups which were discarded because of egg laying are continued as of the last week they were extant.

Finally, the ineffectiveness of the methanol extract of the residue of abdominal wall after extraction in water would be explained on the basis of removal of the material by the first extraction.

The fact that methanol extract of visceral residue was apparently effective, even though the water extract was not, might indicate, as stated above, the existence of an inhibiting substance there different from that in the other two portions, since it seems to be insoluble in water but soluble in methanol. The simpler theory, namely,

that the inhibiting substance is absent in the viscera, seems preferable, but this leaves to be explained the seeming effectiveness of the alcohol extract (series C). The reduced reproductivity in series C can well be due, it is believed, to the unfavorable conditions in that series. First it should be noted that 6 of the 20 groups of this series had died out by the thirteenth week (table 12, C; fig. 1), and 5 by the tenth week, and that 3 other groups had been reduced by death to 4 nymphs or less each by the eighth week. Furthermore, series C has distinctly the highest mortality of the seven series, very strikingly so when the groups which died out are counted in (fig. 5), but definitely so even when only the 14 series which persisted are considered (fig. 4). It seems at least a justifiable hypothesis, therefore, that the failure of numerous groups of series C to produce eggs during the period of the experiment, and the small numbers of supplementaries produced in the series, are direct results of the same unfavorable conditions, presumably incidence of disease, which resulted in the high mortality.

If mortality in series C invalidates the assumption of inhibition, may not similar factors operate to cause the seemingly significant results in series E, B, and D? As for groups dying out, there seems to be no significant difference: series E lost 1 group, as did F and A; D lost 2, as did G; and in B all groups outlived the experiment. As for general mortality (figs. 4 and 5), it will be seen that B has low mortality and therefore conditions within its groups were presumably relatively favorable. In B the low production of eggs (fig. 1) and of supplementaries during the first ten weeks (figs. 2 and 3) must be supposed to be due either to an inhibiting effect of the extract of the head-thorax or to some other unknown factor or factors in the experiment. Aside from C, which stands out as having high mortality, the differences in mortality among the series seem to offer little aid in explaining differences in reproductivity except that the relatively rapid increase in mortality in series E beginning at the eighth week (figs. 4 and 5) raises the question of the possible relation in this series between poor condition and lowered reproductivity.

Another fact which must be considered in evaluating the results is that although the nymphs of all groups were of approximately the same instar they were derived from several stocks. It was assumed that nymphs of different instars have different potentialities for development into supplementaries; it was not expected, however, that nymphs of the same instar but from different colonies would have different potentialities in this regard. More recent investigation (Light, 1942-1943), the details of which are yet to be published, indicates that the first assumption was correct, but also that different colonies show very marked differences in the rate and extent of development of supplementaries in groups of nymphs of a given instar. No record was kept of the origin of the nymphs used in this experiment. There is recorded a note, however, that groups 15-20 of series A, 15-20 of B, 1-10 of C, and 1-10 of D may have been *Zootermopsis nevadensis*. An inspection of table 12, B, D, and C, will show that there is a marked difference between the groups designated and those of the rest of these series. This discrepancy is most obvious when numbers of supplementaries produced are compared, but it is seen also in the numbers of groups which produced eggs.

The results of the 1937 experiment, therefore, although seemingly significant, require confirmation because of the possibility, first, that the differences in recorded reproductivity may be due in part to the fact that the experimental animals were

from different colonies with different potentialities in reproductivity, sometimes even from colonies of different species, and secondly, that they may be due to physiological differences or to disease.

OTHER EXPERIMENTS ON EXTRACT INHIBITION

The importance of grooming in the communal behavior made it seem probable that it served to transmit the inhibiting substance, if such exists, and that therefore this substance is a surface exudate. Extracts of unopened supplementary females were fed, therefore, to test their efficacy in inhibiting or retarding egg laying and the development of supplementaries in groups of nymphs. Four such experiments were planned and initiated at various times. One was early discontinued because of an epidemic of disease.

In two of these experiments (WE and RW) an attempt was made to offset the possible effects of differential mortality by constant replacement of dead termites with individuals from groups of the same history and treatment. Suffice it to say that the result was greatly increased mortality, arising in part presumably from antagonism between the groups and the introduced individuals, and in part from the increase of opportunity for introduction and spread of disease. In each experiment, therefore, the idea of replacement was soon abandoned; not, however, before enough had been done to complicate the evaluation of results.

The first of these experiments (WE) ran from October, 1940, to January, 1941. All animals used as controls or experimentals were from the same colony. One hundred and thirty groups were used, each group consisting of 20 nymphs of the same types and instars: 8 wing-padded nymphs, 1 broad-headed nymph (eighth instar or later), 5 apterous nymphs of the sixth or seventh instar, 2 fifth-instar nymphs, 2 of the fourth instar, and 2 of the third.

Extraction was made from 50 egg-laying females each week by successive treatment with three media: (1) dilute Sørensen's phosphate buffer at pH 7, (2) a mixture of 100 per cent ethyl alcohol and 100 per cent ethyl ether in a ratio of 3:1, and (3) petroleum ether (benzene). The aqueous extraction was continued for 6 hours at 75° F., with frequent agitation. With the alcohol mixture and with the petroleum ether the extraction was continuous, the reproductives being placed in a Gooch crucible attached to a holder near the top of a jar (which was fitted with a Barrett form extraction condenser) and maintained at a temperature which allowed for constant evaporation and recondensation of the medium. Continuous extraction proceeded for four hours with alcohol ether and overnight with petroleum ether. The three extracts were concentrated to the same volume and equal amounts of each of the three were added to the papers fed the experimental series, as follows: The petroleum ether extract was added first and the medium allowed to evaporate completely before the alcohol ether extract was added. When the papers were completely dry again, half the aqueous extract was added. At the middle of the week the rest of it was added.

Two series, of 30 groups each, constituted two different types of control. One series (N, figs. 6-9) consisted of groups of nymphs which were fed paper treated with the three extracting media (it had been indicated by earlier experiments that controls fed extracts of nymphs behaved essentially as did those fed paper only or paper treated with the extracting medium). The second series of controls (P) was

fed as described above, but an egg-producing supplementary pair was added to each group of nymphs. Thus the first series of controls was designed to show the rate and amount of reproductivity in the absence of inhibition; and the second, the degree of inhibition resulting from contact with functional reproductives. The experimental series (W) consisted of 40 groups, each of which each week was fed filter paper treated with the three different extracts, the average available dose being 0.007 of the extract of one reproductive per nymph per day.

Figure 6 gives the results at the sixth, eighth, and twelfth weeks in terms of percentages of groups with supplementaries; figure 7, in terms of percentages of groups with eggs; and figure 8, in terms of percentages of populations which had become supplementaries. The contact-inhibited series (P) is by no means completely inhibited, as figures 6 and 8 show; indeed, contact inhibition is only known to be com-

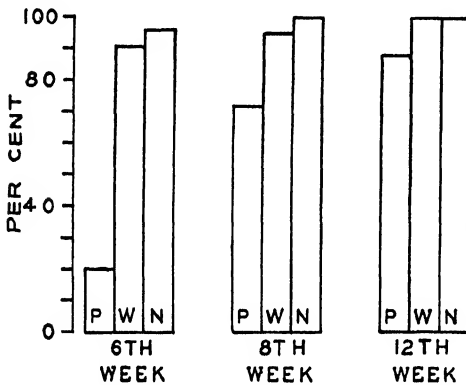


Fig. 6

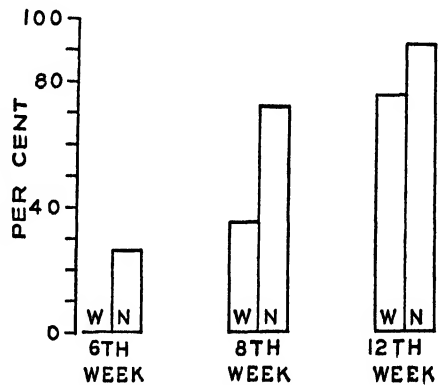


Fig. 7

Fig. 6. Bar diagrams showing groups of the three series of experiment WE which had produced supplementaries at the sixth, eighth, and twelfth weeks, expressed as percentages of the numbers of groups existing at the particular week. Some groups died out during the experiment.

Series N was the uninhibited controls fed paper treated with the extracting media. Series P was fed as was series N, but each group contained a supplementary pair; the series constituted, therefore, a contact-inhibited control. The experimental series (W) consisted of groups of nymphs fed extracts of female supplementaries.

Fig. 7. Bar diagrams showing numbers of groups which had produced eggs by the sixth, eighth, and twelfth weeks in series W and N in experiment WE, expressed as percentages of the groups existing at the particular week. Since the supplementary females introduced into the groups of series P laid eggs from the start, this series is omitted here.

plete when it is unbroken from the inception of the colony (Light, 1942-1943). There is, nevertheless, a marked reduction or retardation in development of supplementaries and in egg laying in the contact-inhibited groups, much greater than any yet produced by means of extracts. When comparison is made between the supposedly extract-inhibited series (W, figs. 6-8) and the controls with respect to time of laying eggs, numbers of groups with supplementaries, and total supplementaries produced, it is seen that there is a slight retardation of the extract-fed groups which might be attributed to the inhibiting influence of the extract. The difference is so slight, however, that its significance would be questionable even if all other factors were equal. It is especially questionable in this experiment because the mortality was very high

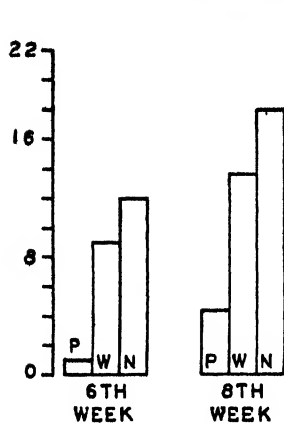


Fig. 8

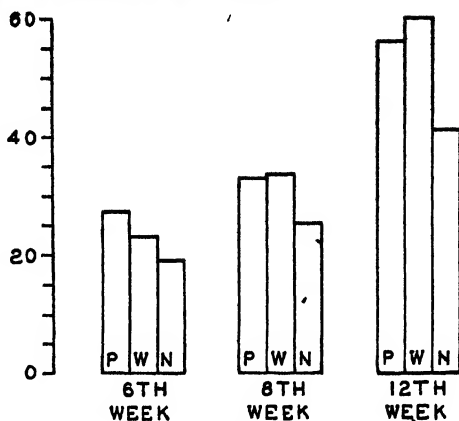


Fig. 9

Fig. 8. Bar diagrams showing the total numbers of supplementaries recorded for groups of the three series of experiment WE extant by the sixth, eighth, and twelfth weeks, expressed as percentages of the original populations of these groups.

Fig. 9. Bar diagrams showing mortality for the series of experiment WE, expressed as percentages of the original populations of the groups existing at the particular week.

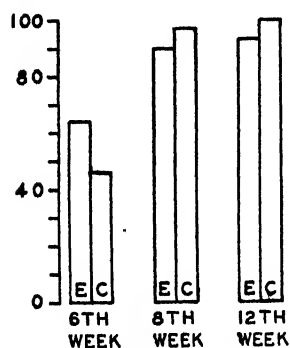


Fig. 10

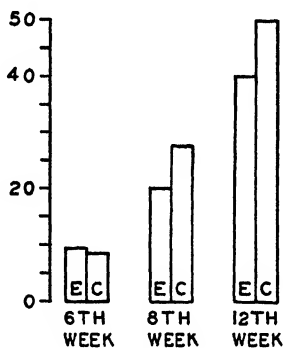


Fig. 11

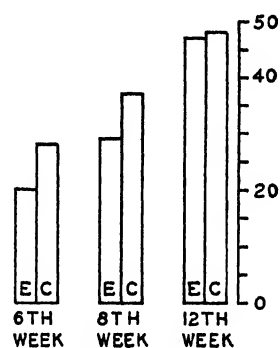


Fig. 12

Fig. 10. Bar diagrams showing numbers of groups which had produced eggs by the sixth, eighth, and twelfth weeks in the series of experiment RW, expressed as percentages of the number of groups existing at the particular week. C was the control series, E the experimentals.

Fig. 11. Bar diagrams showing total supplementaries recorded for the two series of experiment RW as percentages of the original populations of the groups existing at the particular week.

Fig. 12. Bar diagrams showing mortality in experiment RW expressed as percentages of deaths in the original populations of the groups existing at the particular week.

throughout the experiment, and was highest in the experimental series (W, fig. 9) and lowest in the uninhibited controls.

Although the results of this experiment are of interest in relation to contact inhibition, they allow of no conclusions concerning extract inhibition except that the inhibiting effects of the extract, if any, must have been very slight since even though conditions were unfavorable, as indicated by low viability, reproductivity in the experimental series did not fall markedly below that of the uninhibited control.

Experiment RW was a repetition of WE except that extractions were carried out with only two media, (1) dilute Sørensen's phosphate buffer at pH 7.0, and (2) ben-

zene. The ethyl alcohol-ethyl ether extraction was omitted as unnecessary. The contact-inhibited control series was also omitted. The number of egg-laying supplementaries extracted each week was increased to 80, making the average available dosage 0.0143 of one supplementary female per nymph per day for the 40 groups originally constituting the series. The number of groups was reduced considerably during the experiment because some groups were used for replacements early in the experiment and others died out. The theoretical dose therefore correspondingly increased.

Figures 10 and 11 give a summary of the results with respect to reproductivity. Here again the difference, though slight, is in the direction of inhibition. Moreover, as figure 12 shows, the mortality was highest in the control group, in contrast to the conditions in WE. The greater viability in the experimental series serves to increase the seeming significance of the reduced reproductivity in that series.

There remain to be reported the results of one final group of experiments in extract inhibition, experiments SE, SX, and HX. These experiments involved five series all run at the same time, beginning December 24, 1941, and made up of groups of the same composition from the same colony. Each group contained 10 nymphs, of which 2 were wing-padded, 2 of the fifth instar, and 6 of the sixth instar. Two hundred and twenty groups were used, 50 groups in each of the two series SE-S and SE-N and 40 each in HX-S, HX-N, and SX-C.

The 50 groups of SE-S, the experimental series of SE, were fed aqueous surface extracts of whole supplementaries, males and females in equal numbers. Here an attempt was made to simulate normal conditions in the colony, under which all nymphs are exposed to reproductives of both sexes. Extraction was made first for 4 hours at 60° C, the fluid was decanted, fresh water was added, and a second extraction was made for 2 hours at 100° C. Glass-distilled water was used. Since 50 pairs were extracted weekly for feeding SE-S, the average available dose was 0.0143 of the extract from one supplementary pair per nymph per day. The groups of SE-N were fed weekly the extracts of 250 pale nymphs of the third and fourth instars made as for SE-S.

The experimental series of HX (HX-S) was fed weekly the aqueous extracts of the ground-up heads of 40 female supplementaries, an extract which had seemed to have powerful inhibiting effects in the 1937 experiment. The average available dose, therefore, was 0.0143 of the extract from the head of one female supplementary per nymph per day. After grinding, the material was allowed to stand at room temperature for an hour in 10 cc. of double-distilled water, after which the supernatant liquid was decanted. This was repeated and the two fluids were combined, allowed to stand overnight, and then were pressure-filtered and concentrated under vacuum to 9 cc. Like ES-N, HX-S was a series fed extracts made in the same way as for HX-S, but from unpigmented nymphs. One hundred pale third- and fourth-instar nymphs were used weekly. SX-C was a series fed filter paper only, and served as an uninhibited control for both extract-feeding experiments (SE-S and HX-S), and SE-N and HX-N were intended as controls for possible effects resulting from feeding extracts of ordinary nymphs prepared by the two methods.

Significant parts of the results in these five series (table 15) are presented together since they were run at the same time and since the groups were of the same composition and from the same colony. These results offer little satisfactory evidence for

extract inhibition. The supposedly completely uninhibited controls (SX-C) which were fed paper only were consistently lowest in reproductivity as expressed in terms of total supplementaries produced, and nearly so in terms of groups with eggs, which may be at least partly accounted for by unfavorable conditions indicated by relatively high mortality (table 15), but which certainly renders it difficult to derive evidence for extract inhibition from the results of this experiment.

TABLE 15

SIGNIFICANT SAMPLES FROM THE RESULTS OF EXPERIMENT SE-HX

(Results presented as for 40 groups per series. Series arranged in order of decreasing reproductivity. Mortality is expressed in percentages of the original populations.)

Series	Groups with eggs				Total supplementaries			
	All groups considered		Living groups only		All groups		Living groups	
	14th week	16th week	14th week	16th week	14th week	16th week	14th week	16th week
SE-S.	13.6	19.2	14.78	20.87	84.0	95.2	89.56	101.74
SE-N.	12.8	17.6	13.91	18.66	77.6	92.8	84.35	99.55
HX-N.	11.0	11.0	11.30	11.30	82.0	85.0	84.10	87.20
SX-C.	7.0	7.0	7.20	7.20	59.0	68.0	59.50	69.70
HX-S.	7.0	7.0	7.00	7.00	91.0	97.0	91.00	97.00

Series	Mortality				Number of groups which had died out		
	All groups		Living groups				
	14th week	16th week	14th week	16th week	14th week	16th week	20th week
SE-S.	29.80	32.20	23.69	25.43	4	4	8
SE-N.	35.80	39.40	30.00	32.66	4	5	5
HX-N.	28.25	33.50	26.41	31.80	1	1	
SX-C.	30.25	37.25	28.46	35.64	1	1	2
HX-S.	22.75	28.25	22.75	28.25	0	0	

If there is any evidence for extract inhibition, it arises from the contrast between the results in HX-S and those in the two nymph-fed control series SE-N and HX-N (see table 15). However, results in HX-S are so closely similar to those in the control series SX-C that no inhibition can be postulated unless the distinctly higher mortality in SX-C be held to indicate unfavorable conditions causing an abnormally low reproductivity in that series.

Series SE-S, fed surface extracts of whole supplementary pairs, far from showing any inhibition, was highest both in groups with eggs and in number of supplementaries. A close second to it was control series SE-N, fed surface extracts of male and female unpigmented nymphs. These results are the more striking because these two groups suffered high mortality (table 15), as indicated both by the number of groups which died out and by the percentage of the original population which died. When, however, only those groups which survived for the duration of the experiment are considered (table 15), the reproductivity of these series remains the highest, but their relative mortality is seen to be low, distinctly the lowest in SE-S. We are left with the question, then, whether the higher reproductivity in SE-S and SE-N was due (1) to the more favorable conditions prevalent in most of the groups, although

conditions were distinctly unfavorable in the groups which did not survive, or (2) to the fact that the series were fed extracts of both males and females (the advisability of further experimentation seems indicated here), or (3) to chance differences in original constitution, in treatment, and in the incidence of disease.

Several features of the results indicate that conditions were very unfavorable in this experiment. Thus, mortality was high in general (table 15). A considerable number of groups died out before the end of the experiment, and the groups were very slow to lay eggs, many of them failing to lay at all within the period of the experiment. The unfavorable conditions were no doubt largely due to the small size of the groups used, 10 nymphs. Experience has shown that such small groups have difficulty in achieving social integration and in controlling the environment. Furthermore, the differences between control series with regard to viability and reproductivity suggest that there was differential incidence of disease or significant difference in handling the series.

In evaluating the results of this experiment it should be kept in mind that the colony here used was a most peculiar one, the largest we have encountered, consisting of 12,785 recorded individuals, perhaps a mixture of two or more colonies, and headed by about 30 ordinary supplementaries and a number of soldier-neotenic intergrades (the reproductive soldiers of Heath, 1927).

GENERAL RESULTS

Altogether I have presented a considerable number of instances of reduced reproductivity in series fed various extracts of supplementary reproductives as compared with control series. In some instances this lowered reproductivity is correlated with low viability. In others, however, the reverse is true, relatively low reproductivity being associated with relatively low mortality. It is these instances (table 16) which supply the evidence, such as it is, for the existence of inhibiting substances and extract inhibition.

SUMMARY AND CONCLUSIONS

1. An inhibiting substance, an ectohormone or social hormone, produced by functioning termite reproductives and presumably spread through the colony by grooming or by stomodaeal and proctodaeal feeding, has been postulated to explain the absence or relative scarcity of supplementary (neotenic) reproductives in colonies headed by primary reproductives, and their appearance in orphaned colonies and in isolated groups of nymphs.

2. Experiments by Castle (1934) and by Ridder (unpublished) which seemed to supply evidence in support of this theory are presented and discussed. This evidence took the form of delay in egg laying and delay in the appearance of pigmented supplementaries in groups of nymphs fed extracts made from opened female supplementaries (Castle) or ground-up male supplementaries (Ridder) with 70 per cent ethyl alcohol or with ether.

3. Numerous experiments carried out more recently under my direction and designed to supply decisive evidence with respect to extract inhibition are presented in such detail as the significance of their results seems to justify. These experiments involved many thousands of individuals in hundreds of groups. Various methods of extraction were tested and varying theoretical dosages administered.

TABLE 16
SUMMARY OF INSTANCES OF SEEMING OR ACTUAL PARTIAL INHIBITION OF REPRODUCTIVITY RESULTING FROM FEEDING OF EXTRACTS

Series	Material extracted	Extracting medium	Recorded reproductivity	Viability compared to controls	Conclusions in terms of inhibition	Remarks
Castle's experiment 1	Slit female supplementaries	70 per cent ethyl alcohol	Low	No information	Probably inhibition	Might have been due to unfavorable conditions in experiments
Castle's experiment 2	Slit female supplementaries	Ether	Low	No information	Probably inhibition	Same as above
		70 per cent ethyl alcohol	Low	No information	Probably inhibition	Same as above
Ridder's experiment	Ground male supplementaries	Ether	Very low for males and egg laying	Little information	Seemingly inhibition	May have been due to low viability. Findings questioned
EX-S	Slit female supplementaries	Ether	Low	High	Inhibition strongly indicated	
1937-E	Ground head and thorax of female supplementaries	Water	Very low	High, but sharply lowered in later period	Inhibition indicated	Results clouded by fact that material came from different colonies or even species
1937-B	Head-thorax	Methanol	Low	High	Some inhibition indicated	Material possibly from two species
1937-D	Abdominal wall	Water	Low	Intermediate	Possibly inhibition	Material possibly from two species
RW-E	Entire female supplementaries	Combinations of extracts in (1) 100 per cent ethyl alcohol, (2) alcohol and ether, and (3) benzene	Somewhat lower than controls	High	Possibly inhibition	
HX-S	Ground heads of female supplementaries	Cold water followed by water at 100° C.	Lower than some of the controls	High	Possibly inhibition	

4. In a number of experiments the series fed extracts showed some degree of retardation or reduction of reproductivity as compared with control series and measured in terms of the time to egg laying, the number of groups laying eggs, the number of supplementaries produced, and the time at which they appeared.

5. In general it must be said that, although the results are indicative of inhibitory effects from the extracts, they cannot be considered to supply conclusive evidence in support of the ectohormonal theory of inhibition of reproductivity, an inhibition which is typically complete in nature in colonies headed by primary reproductives.

6. In no experiment was complete inhibition obtained.

7. In only one of them (1937, E) did the seeming inhibition approach or equal the reduction of reproductivity obtained by introducing a functioning supplementary pair into each group of one of the control series of one of the experiments (WE-P).

8. In none of the experiments, indeed, was the difference in reproductivity between the extract-fed experimentals and the uninhibited controls of a magnitude to preclude its having been caused by the uncontrolled variables of composition, condition, and treatment which give rise to the differences in reproductivity between series of the same origin, supposedly of the same constitution, and given, supposedly, identical treatment (see series LC-1-LC-3, tables 4-6).

9. In some experiments mortality was so high as to bring into question the significance of the results. In others, however, notably EX, mortality was low and there were no indications of unfavorable conditions.

10. In some experiments mortality was higher in the experimentals than in the controls, suggesting that the lowered reproductivity there might have been due to physiological or pathological conditions and not to inhibitory effects of the extracts (see the 1937 experiment, series C).

11. In a number of experimental series, however, the mortality was lower than in the controls. Under such circumstances reduced reproductivity seems to have significance as indicating an inhibitory effect from the extracts (see table 16 for a summary of such instances).

12. Again, in the 1937 experiment the significance of the results is clouded by the fact that the nymphs used were from different colonies or colony fragments with different histories and, as we now know (Light, 1942-1943), different potentials of reproductivity.

13. In most of the experiments, however, all animals were from one colony and the groups were of the same composition, and in some, such as EX, neither mortality nor any other obvious feature of the situation explains the lower reproductivity of the groups fed extracts of supplementaries.

14. Furthermore, even where, as in the 1937 experiment, some of the results may be due to differences in potentials of reproductivity and to differences in physiological conditions, as indicated by mortality, the degree of difference between experimentals and controls is great enough to indicate inhibition, especially in the series fed water extracts of the head-thorax.

15. When the results of all these experiments are considered, it is seen that in most instances the series fed extracts of reproductives either showed a lower reproductivity than the controls, or a viability sufficiently higher to allow for the belief that the difference in condition between the controls and the experimentals was great enough to obscure the inhibiting effects of the extracts.

16. The regular occurrence of more or less definite indications of inhibition in the form of retarded or decreased reproductivity leads to the presumption that contact inhibition is accomplished by means of a substance. The ectohormonal theory, therefore, while not proved, is supported by the trend of the evidence.

17. Groups of nymphs isolated from a colony, such as have been utilized heretofore in the study of this problem, have many disadvantages. The nymphs have undergone the shock of breaking thoroughly established social relations, they have been subjected to the vicissitudes of extensive handling, and they must endure the unfavorable conditions which exist during the period of readjustment and social reintegration. During this period of readjustment, group action fails in greater or less degree, with the result that control of the environment is imperfect, especially with respect to moisture. Microörganisms therefore often grow uncontrolled. This and other factors make for highly variable mortality and for great irregularity in physiological functioning if reproductivity may be taken as a measure. Groups often die out entirely, sometimes early in the experiment, sometimes, perhaps always, from disease. Other groups become greatly reduced in number but do not die out, and of these reduced groups some develop supplementaries and lay eggs quite normally. On the other hand, highly viable groups occasionally fail to lay eggs or, sometimes, even to develop supplementaries. In general, the irregularities and abnormalities arising from the laboratory method employed as described above are such as to tend to obscure the significance of the results.

18. Several facts seem to indicate that if reproductives produce an inhibiting substance, it is produced in small amounts, but continuously, and that it is effective when continually received, even though in minute amounts. Inhibition is complete only in primary colonies which have been continuously in the presence of the reproductives. Experimental contact inhibition among groups of nymphs isolated from a mature colony is variable and far from complete, presumably because of a period of readjustment during which the nymphs do not associate closely with the introduced reproductives. Even so, it is more effective than anything obtained by feeding extract. In our extract-feeding experiments the actual dosage of the hypothetical inhibiting substance has probably been low for one or more of several reasons: (1) each supplementary presumably contains but little of the material; (2) perhaps the methods of extraction are not effective, or alter some or all of the substance concerned; (3) the extracted substance may deteriorate rapidly, with the result that little active material is ingested and that during extensive periods between feedings no effective material is eaten. Higher dosage of extract may be obtained either by increasing the ratio of supplementaries extracted to nymphs fed, as by using more supplementaries or smaller nymphal groups, or by feeding at more frequent intervals.

19. Incipient colonies would seem to offer the best opportunity for a definitive test of the existence of inhibiting substances. Each group would then be a normally integrated social entity, and social disruption would be reduced to a minimum since only the primary pairs would need to be handled. The small size of each group and the small size of the component individuals would make it possible to administer relatively more massive doses of extract without requiring too great an increase in supplementary-producing stocks. Finally, it seems probable that the length of time during which extracts must be fed can be reduced materially by making extract

feeding an interlude between contact inhibitions, thereby allowing for still more massive doses without increasing supplementary-producing stocks. It should be possible to determine by experiment how long a colony must be isolated from the primaries in order to insure the development of supplementaries after the primaries are later returned. If colonies fed extracts during this period of isolation and perhaps for a short period of readjustment after return of the primaries fail to produce supplementaries, whereas comparable control colonies fed no extracts do produce supplementaries, the existence of an inhibiting substance will have been proved. Experiments of this type are projected for the next swarming season.

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